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The Relationship between Dietary Deficiency and the Occurrence of Papillary Atrophy of the Tongue and Oral Leukoplakia*

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There is now considerable evidence to show that papillary atrophy of the tongue frequently is the result of a deficient ingestion of vitamin B complex (11, 13, 21). In some instances, it is stated, such glossal lesions can be treated successfully by the administration of nicotinic acid alone or with riboflavin; in others the use of the whole vitamin B complex in a crude form apparently is needed in order to effect restoration of the normal glossal surface.

The high incidence with which papillary atrophy of the tongue is noted in this hospital to be associated with leukoplakia of that organ or of other areas of the mouth immediately suggests the possibility that oral leukoplakia likewise is the result of a deficient ingestion or utilization of essential dietary constituents. Numerous suggestions concerning the etiology of oral leukoplakia already have been advanced, with syphilis and the excessive use of tobacco (7) particularly suspect. Yet many patients with the disease neither suffer from syphilis nor use excessive amounts of tobacco, and therefore these factors cannot be considered in themselves to be the cause of the disorder.

A need for a better understanding of the cause of both glossal atrophy and oral leukoplakia is especially important because of the possible precancerous nature of these lesions. Cancer frequently is seen in association with leukoplakia, and has been observed to develop in it.

During the past year it has been possible to investigate in a large group of patients with glossal papillary atrophy or oral leukoplakia the relative etiologic importance of tobacco, syphilis, and dietary deficiencies. These patients were examined further for other pathological changes which might be associated with their oral lesions, and were observed for from 6 to 10 months, a period during which they were treated with large amounts of brewer's yeast as a source of vitamin B complex.

CLINICAL MATERIAL

The clinical material studied comprised 3 groups of patients who attended the out-patient department of the Head and Neck Service of the Memorial Hospital. In addition, as control material the following subjects were employed: (a) a group of 25 normal adults; (b) 20 women with cancer of the breast; (c) 12 males and 13 females with cancer of the stomach; and (d) 10 men and 5 women with leukemia. These individuals were studied only to ascertain the incidence among them of syphilis, excessive smoking, and histories of dietary deficiency.

Of the experimental subjects, the first group consisted of 17 adults with papillary atrophy of the tongue. Of these, 6 were male and 11 were female. The atrophic changes were observed to affect from one-fourth to all of the organ surface, and in 4 instances were associated with small ulcers of the denuded areas.

The second group comprised 29 adults with leukoplakia of the tongue, buccal mucosa, gingivae, or inner surfaces of the lips. Of the 29 patients, 24 were males and 5 were females. The size of their lesions varied from discrete areas 0.5 cm.^{2} to extensive involvement of both buccal mucosae, most of the tongue, and gums. In 11 of the 29, the lesion was

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limited to the tongue alone. The leukoplakia varied in thickness from thin filmy lesions, usually seen on the buccal mucosae, to thick, horny layers. Of the 29 patients, 7 also had ulcerated areas in the midst of, or adjacent to, their oral lesions.

The third group was composed of 21 patients who had both papillary atrophy of the tongue and leukoplakia of the mouth. Of these, 13 were males and 8 were females. The glossal papillary atrophy and leukoplakic lesions appeared much the same as those observed in group 2. Ulcerated areas in the midst of the leukoplakic areas were found in 4 instances.

METHODS

The criteria used for the presence of hepatic insufficiency in the patients studied were (a) the existence of hepatomegaly; (b) a concentration of serum protein of 6.5 gm. per cent or less, by the method of Weech (23); (c) a ratio of free to esterified cholesterol of more than 1:2, by the method of Schoenheimer and Sperry (18); (d) a serum bilirubin of 1.0 mgm. per cent or more, by the method of Sofer and Paulson (20); (e) a mean corpuscular volume of erythrocytes greater than 94 μ^3 by the method of Wintrobe (16); and (f) a urinary excretion of glucuronates of less than 300 mgm. per day, by the method of Maugham, Evelyn, and Browne (14). These criteria and the basis for their use to indicate hepatic insufficiency have been discussed in a previous communication (1).

To determine whether or not the patients suffered from a general avitaminosis, their plasma levels of vitamin A were measured by the technic of Clausen and McCoord (5) and their excretions of riboflavin in at least one 3-day collection of urine by the method of Ferrebee (8). The plasma levels of vitamin A in normal males have been found to range from 132 to 208 U.S.P. units per cent, and in females from 103 to 180 U.S.P. units per cent. The excretion of riboflavin in the urine of normal individuals varies from 275 to 2,000 μgm. per day.

The Kahn and Klein serological tests were used to establish the diagnosis of syphilis. In several instances those which were positive were confirmed by the routine Wassermann examination.

The existence of free hydrochloric acid in the stomach was ascertained by the titration technic (14). Fasting gastric contents were aspirated both before and one-half hour after the intramuscular injection of 2 mgm. of histamine. Both specimens were titrated with sodium hydroxide to the methyl orange end point.

RESULTS

The results of this investigation are presented in three parts: 1. Results of a study of the factors which might be responsible for the production of papillary atrophy of the tongue and of oral leukoplakia. 2. Results of a study of the anatomical and physiological changes associated with the oral lesions. 3. The results of the administration of large amounts of yeast to the patients studied.

1. A Study of the Factors Which Might Be Responsible for the Production of Papillary Atrophy of the Tongue and Oral Leukoplakia

The explanations which have been advanced for the cause of glossal papillary atrophy and of oral leukoplakia are (a) syphilis, (b) the excessive use of tobacco, and (c) dietary deficiency. It was necessary to ascertain, therefore, the incidence of these suggested etiologic mechanisms in the patients studied. In addition, the fact has been demonstrated that the existence of hepatic dysfunction in patients on normal diets frequently interferes with the proper utilization of vitamins and other essential dietary factors (1, 4). Such individuals, therefore, may suffer from a conditioned vitamin deficiency. Acordingly, it was desirable to determine also the incidence of (d) hepatic insufficiency in the patients included in this investigation.

a. *Syphilis.*—Determined by serologic means, the incidence of syphilis among the out-patients of this hospital who were used as controls was 5 per cent of 20 who attended the Breast Clinic, 8 per cent of 25 in the Gastric Clinic, and 7 per cent of 15 in the Medical Clinic. The incidence of syphilis among 25 apparently normal persons seen in this hospital was 4 per cent.

Of 17 patients with papillary atrophy of the tongue, 15 were examined for serologic evidence of syphilis, and all were found to be negative. Of 29 patients with leukoplakia of the mouth, Kahn and Klein tests were done in 27 and found positive in 3 (or 11 per cent). Of the 21 patients who had both glossal papillary atrophy and oral leukoplakia, only 1 (or 4.5 per cent) had positive Kahn and Klein tests (Table I). All of these patients with syphilis at the time of this investigation had received adequate antiluetic care for 2 or more years without any remission of their glossal or oral lesions. It would appear, therefore, that the incidence of syphilis in patients with either glossal papillary atrophy or oral leukoplakia is not significantly higher than those of the control groups. Furthermore, if the oral and lingual lesions in those patients with syphilis were due to that disorder, those lesions must be considered as permanent effects of the syphilitic process, for they had not subsided during the administration of antiluetic therapy for at least 2 years.

b. *Excessive use of tobacco.*—The use of tobacco was considered to be excessive when the patient smoked per day more than 25 cigarettes, 10 cigars,

or 10 pipesful for 2 or more years before the appearance of the oral or lingual lesion. Histories of this degree of excessive smoking were obtained from 18 per cent of the patients with glossal papillary atrophy, 55 per cent of those with oral leukoplakia, and from 46 per cent of those who had both lesions (Table I).

In contrast, the incidence of excessive smoking in a group of 25 patients with leukemia and of 25 normal adults, used as controls, was found to be only 8 per cent of each group. Therefore, it must be concluded that the use of tobacco by the patients with glossal or oral lesions *was* abnormally great.

c. *Inadequate diets.*—Dietary histories were obtained from all of the patients studied. The dietary intake of a patient was considered to be inadequate when for at least 3 years it did not include a daily 4-ounce portion of fresh or cooked vegetables, cereals, or fruits, or from 4 to 6 ounces of meat or fish twice a week. By these standards it was ascertained that inadequate diets were taken by only 4 of 25 (16 per cent) women with cancer of the breast, 13 of 51 (24 per cent) patients with cancer of the gastrointestinal tract, 2 of 13 (15 per cent) patients with atrophic gastritis, and 3 of 25 (12 per cent) apparently normal persons.

Although 2 of these 3 groups used as controls had gastrointestinal disease, their incidence of inadequate diet was considerably less than that of the patients with lingual or oral lesions. Of those with papillary atrophy of the tongue, 10 (59 per cent) had diets grossly deficient in animal protein, vegetables, or both. Sixteen (55 per cent) of those with oral leukoplakia, and 13 (61 per cent) of those with the combined lesions, had been on similarly inadequate diets (Table I).

Furthermore, of the 17 patients with glossal papillary atrophy, 4 had abnormally low plasma levels of vitamin A, and 2 others had abnormally low urinary excretions of riboflavin. Of these 6 patients with proved deficiency, all but 1 had been on grossly inadequate diets.

Likewise, of the 29 patients with oral leukoplakia, 6 had low plasma levels of vitamin A, 7 others low urinary outputs of riboflavin, and 1 had both a low plasma level of vitamin A and low urinary excretion of riboflavin. All but 2 of these 14 patients had been on grossly deficient diets. Finally, of the 21 patients with combined lesions, 6 had low plasma levels of vitamin A, 1 had a low urinary output of riboflavin, and 1 had low values for both plasma vitamin A and urinary riboflavin excretion. Of these 8, 7 had taken inadequate diets (Table II).

The incidence of a specific deficiency of either vitamin A or B_2 (riboflavin) probably is too low to be considered etiologically significant *per se* in the production of glossal papillary atrophy or oral leukoplakia. In almost all instances the low values of vitamins A and B_2 could be explained by the fact that these patients took generally inadequate diets. Furthermore, it is to be noted that of the 17 patients with papillary atrophy of the tongue, 6 had been on deficient diets and yet had normal plasma levels of vitamin A and urinary outputs of riboflavin. Likewise, of the 29 patients with oral leukoplakia and the 21 with combined lesions, 6 of each group gave histories of deficient diets and had normal values of plasma vitamin A and urinary excretions of riboflavin.

Table I: The Per Cent Incidence of Syphilis, the Excessive Use of Tobacco, and Dietary Deficiency (Simple or Conditioned) among Normal Individuals and Patients with Oral Precancerous Lesions

	Syphilis	Excess tobacco	History of dietary deficiency	History of dietary deficiency or gastric achlorhydria	History of dietary deficiency, gastric achlorhydria, or hepatic insufficiency
17 Patients with papillary atrophy of the tongue	0.0	18	59	76	82
29 Patients with oral leukoplakia	11.1	55	55	61	65
21 Patients with combined lesions	4.5	46	61	81	81
25 Normals	4.0	8	12	0	16

It would appear, therefore, that the incidence of simple dietary deficiency in the patients studied *was* abnormally high and in many instances was confirmed by the low plasma levels of vitamin A and urinary output of riboflavin. It was next important to ascertain whether or not these patients also might suffer from other disorders which might condition the existence in them of a dietary deficiency. The 2 disorders of this nature which were sought for were gastric achlorhydria and hepatic insufficiency.

Gastric achlorhydria can result in a malabsorption of essential dietary constituents (3), and therefore, in order to appraise properly the etiologic importance of dietary deficiency in the production of papillary atrophy of the tongue or oral leukoplakia, the incidence of gastric achlorhydria in the patients studied must be ascertained.

Of the 17 patients with papillary atrophy of the tongue, complete anacidity was found in 7 (41 per cent). Three of these 7 had taken adequate diets, and thus it is probable that they might have suffered from

TABLE II: THE INCIDENCE OF DIETARY DEFICIENCY, VITAMIN DEFICIENCY, AND GASTRIC ACHLORHYDRIA IN THE PATIENTS STUDIED

PATIENTS WITH GLOSSAL PAPILLARY ATROPHY

Patient	Sex	History of inadequate diet	Plasma vitamin A U.S.P. units per 100 ml.	Urinary excretion of riboflavin μgm. per day	Gastric achlorhydria
T.W.	F	0	*88* *	1,170	+
L. O.	F	0	195	365	+
H. K.	F	0	155	460	0
S. U.	F	+	121	*106*	+
M. R.	F	+	175	460	0
G. J.	F	+	*102*	331	0
G. O.	F	+	138	278	+
C. F.	F	0	105	488	0
G. M.	F	+	175	*115*	0
C. B.	F	+	231	475	0
A. D.	F	0	130	817	0
I. R.	M	+	138	580	+
G. K.	M	0	132	...	0
J. F.	M	+	*90*	1,455	0
S. J.	M	+	150	406	0
T. C.	M	+	*51*	1,077	+
I. L.	M	0	242	437	+

PATIENTS WITH ORAL LEUKOPLAKIA

Patient	Sex	History of inadequate diet	Plasma vitamin A U.S.P. units per 100 ml.	Urinary excretion of riboflavin μgm. per day	Gastric achlorhydria
A. J.	F	+	190	...	0
D. L. B.	F	+	115	*256*	0
D. L.	F	+	*98*	330	+
H. G.	F	+	170	1,938	+
J. B.	F	0	170	698	0
I. S.	M	+	*109*	*203*	+
E. S.	M	+	215	*268*	0
J. B.	M	0	210	771	0
A. P.	M	+	185	*258*	..
A. K.	M	+	132	465	0
H. K.	M	0	188	...	+
M. M.	M	+	223	*131*	..
I. C.	M	0	305	...	0
I. M.	M	+	212	*101*	..
J. R.	M	+	138	2,180	0
B. D.	M	+	*122*	985	0
J. F.	M	0	163	1,662	0
L. F.	M	0	*110*	1,738	0
W. J.	M	0	133	680	+
J. W.	M	0	145	400	..
F. N.	M	+	*51*	...	0
W. N.	M	+	169	*213*	+
J. S.	M	0	210	400	0
W. R.	M	0	176	716	0
J. S.	M	0	138	401	0
D. S.	M	+	217	488	0
G. Y.	M	0	195	709	0
J. R.	M	+	*112*	566	0
A. B.	M	0	245	*118*	0

PATIENTS WITH COMBINED LESIONS

Patient	Sex	History of inadequate diet	Plasma vitamin A U.S.P. units per 100 ml.	Urinary excretion of riboflavin μgm. per day	Gastric achlorhydria
A. S.	F	+	215	2,965	0
D. B.	F	0	142	560	0
B. B.	F	+	160	...	0
A. B.	F	0	...	...	+
A. C.	F	0	269	2,790	+
C. B.	F	+	*98*	593	0
D. M.	F	+	*31*	*138*	0
A. K.	F	+	136	1,520	0
F. W.	M	+	129	775	0
P. K.	M	+	140	1,080	0
C. K.	M	+	140	*104*	0
L. D.	M	+	*126*	841	0
B. H.	M	+	*90*	668	+
T. R.	M	+	134	1,640	+
A. B.	M	0	193	640	+
S. J.	M	0	331	467	0
E. K.	M	+	381	352	0
E. McG.	M	0	*85*	712	+
P. N.	M	+	*86*	...	0
J. S.	M	0	140	544	0
D. S.	M	0	269	797	0

* Values in italic are abnormally low.

a conditioned dietary deficiency. Therefore, the number of patients of this group who conceivably suffered from inadequate dietary intake or absorption would be raised from 10 (59 per cent) to 13 (76 per cent) (Table I).

Likewise, of 29 patients with oral leukoplakia, gastric analysis in 25 revealed that 6 (24 per cent) had complete achlorhydria. Two of the 6 had normal dietary histories, but because of their achlorhydria probably had defective absorption from their gastrointestinal tracts. Such defective absorption would raise the number of patients of this group with dietary deficiency, simple or conditioned, from 16 (55 per cent) to 18 (61 per cent).

Finally, of the 21 patients with the combined oral and lingual lesions, 7 (33 per cent) had gastric achlorhydria, and of those 7, 4 had been on normal diets. Thus, of the 21 patients, 17 (81 per cent) might be considered to have probable dietary deficiencies.

In summary, a high percentage of patients with either atrophy of the glossal papillae or oral leukoplakia, probably suffer from a lack of dietary factors. The evidence for this conclusion is based primarily upon the dietary histories obtained from these patients, and the observation that 42 per cent of those who had taken inadequate dietaries also had either abnormally low plasma levels of vitamin A or low urinary excretions of riboflavin. The fact that several patients, who apparently had taken normal diets, suffered from gastric anacidity conceivably could have resulted in a conditioned dietary deficiency in those instances. Certainly the existence of gastric achlorhydria in these individuals whose diets were grossly inadequate must have aggravated their dietary deficient states.

d. *Hepatic dysfunction.*—Since it appears that the existence of hepatic dysfunction can result in the inability of an individual to utilize properly vitamins (1), carbohydrate (12), and proteins (4), it was necessary to ascertain both the existence and the extent of hepatic insufficiency among the patients studied. The criteria used in this investigation for the existence of hepatic dysfunction were: 1. an increased size of the liver, 2. a serum bilirubin of 1.0 mgm. per cent or more, 3. a serum protein of 6.5 gm. per cent or less, 4. a ratio of free to esterified cholesterol of more than 1:2, 5. a mean erythrocyte volume greater than 94 μ^3, and 6. a urinary output of glucuronates less than 300 mgm. per day. The basis for the use of these criteria has been discussed in a previous communication (1). Since it has been demonstrated (1) that none of 25 normal individuals had 3 or more of these abnormalities, it seemed reasonable to assume that the patients included in this investigation who did have 3 or more abnormalities of hepatic function had a significant degree of hepatic insufficiency.

Of the 17 patients with papillary atrophy of the tongue, 6 had no abnormal hepatic function, 5 had 1, 4 had 2, and only 2 had as many as 3 hepatic abnormalities. Of the last 2 individuals, 1 had taken a normal diet and had a normal amount of gastric hydrochloric acid (Table III).

Of the 29 patients with oral leukoplakia, 13 had no evidence of hepatic insufficiency, 12 had only 1 hepatic abnormality, 1 had 2, and 3 had 3 or more signs of abnormal liver function. Only 1 of these last 3 individuals had been on a normal diet and had free hydrochloric acid in his stomach.

Finally, of the 21 patients who had both papillary atrophy of the tongue and oral leukoplakia, 10 had no signs of hepatic dysfunction, 3 had 1, 5 had 2, and 3 had 3 or more hepatic abnormalities. All of the last 3 had histories of grossly inadequate diets.

If, as the evidence suggests, a dietary deficiency might have been produced in the patients included in this study because (a) they had taken an inadequate diet, (b) they had gastric achlorhydria, or (c) they had hepatic insufficiency, then a dietary deficiency conceivably existed in 14 (82 per cent) of the 17 patients with papillary atrophy of the tongue; in 19 (65 per cent) of the 29 patients with oral leukoplakia; and in 17 (81 per cent) of the 21 patients with the combined lesions (Table I).

2. The Pathological Changes Associated with the Existence of Papillary Atrophy of the Tongue or Oral Leukoplakia

a. *Gastrointestinal tract.*—Numerous symptoms, apparently associated with the gastrointestinal tract, were described by the patients in this study, and strongly suggested that their oral or lingual lesions were only a part of a generalized gastrointestinal disorder. For this reason, a careful investigation of the gastrointestinal symptoms and signs was made whenever possible.

The most common complaint of these patients was soreness of the mouth or tongue. Of the 17 patients with papillary atrophy of the tongue, 12 (71 per cent) had glossodynia for from 2 months to 15 years before the appearance of any lesions. This symptom was always mildly progressive, and in only 2 instances was the glossodynia subject to short periods of spontaneous remission (Table IV).

Sore mouths, especially after the ingestion of spiced or hot foods, were noted by 14 of 29 (49 per cent) patients with oral leukoplakia, and by 13 of 22 (60 per cent) patients with the combined lesions.

Of the patients with papillary atrophy of the tongue, 7 (41 per cent) had long-standing histories of dysphagia, 7 (41 per cent) complained frequently of postprandial nonradiating, epigastric pain, and 5 (32 per

TABLE III: THE INCIDENCE OF ABNORMALITIES OF HEPATIC FUNCTION IN THE PATIENTS STUDIED

PATIENTS WITH GLOSSAL PAPILLARY ATROPHY

Patient	Hepatomegaly	Serum bilirubin mgm./100 ml.	Serum protein G/100 ml.	Cholesterol /esters mgm./100 ml.	MCV μ^3	Glucuronates mgm./day	Patient considered to have abnormal degree of hepatic insufficiency
A. W.	o	0.95	7.4	81/155	*105*	492	o
L. O.	+	0.60	7.3	*79/138*	80	455	o
H. K.	o	*1.13**	7.3	69/171	72	435	o
S. U.	+	*1.13*	6.9	*39/71*	83	...	+
M. R.	+	0.75	7.2	*54/91*	79	680	o
G. J.	o	0.63	7.0	....	79	538	o
G. O.	+	0.63	7.3	28/60	75	*283*	o
C. F.	o	0.65	7.4	53/160	74	*218*	o
G. M.	o	0.65	..	....	*101*	*63*	o
C. B.	o	0.45	7.1	65/194	87	624	o
A. D.	+	*1.25*	6.8	64/147	90	*119*	+
I. R.	o	0.63	7.3	88/209	86	667	o
G. K.	o	0.63	6.6	85/175	83	...	o
H. F.	o	...	6.6	....	89	415	o
C. J.	o	0.65	7.7	*39/65*	82	392	o
T. C.	o	0.70	7.9	68/156	80	*281*	o
I. L.	o	0.90	6.9	44/125	77	777	o

PATIENTS WITH ORAL LEUKOPLAKIA

Patient	Hepatomegaly	Serum bilirubin mgm./100 ml.	Serum protein G/100 ml.	Cholesterol /esters mgm./100 ml.	MCV μ^3	Glucuronates mgm./day	Patient considered to have abnormal degree of hepatic insufficiency
A. J.	o	*3.50*	8.2	82/181	81	...	o
D. L. B.	o	0.45	7.2	....	..	*270*	o
D. L.	+	*1.75*	8.5	*55/102*	72	378	+
H. G.	o	0.45	6.9	62/137	82	380	o
J. B.	o	0.90	7.8	....	83	379	o
I. S.	+	*2.50*	5.7	*82/98*	70	*183*	+
E. S.	o	0.75	6.7	60/151	*100*	324	o
J. B.	o	0.95	7.3	64/144	70	*179*	o
A. P.	o	0.45	6.6	51/199	81	455	o
A. K.	o	*1.25*	6.5	67/208	88	820	o
H. K.	o	0.60	7.0	51/128	75	...	o
H. M.	o	0.60	6.9	55/129	80	342	o
I. C.	o	...	7.6	85/257	92	605	o
I. M.	..	0.90	6.9	48/128	88	*234*	o
J. R.	o	...	6.9	66/155	90	*290*	o
B. D.	o	*1.15*	7.0	....	82	403	o
J. F.	o	0.75	7.4	....	...	458	o
L. F.	o	0.60	7.0	66/150	95	475	o
W. J.	o	0.90	6.7	58/145	75	*283*	o
J. W.	o	...	6.9	....	80	422	o
F. N.	+	0.90	6.9	68/164	83	436	o
W. N.	o	0.65	7.6	70/151	75	495	o
J. S.	o	*1.25*	*6.1*	100/300	76	*278*	+
W. R.	o	0.80	..	....	94	346	o
J. S.	o	0.90	..	....	90	907	o
D. S.	o	0.90	7.5	....	73	506	o
G. Y.	o	0.75	6.8	57/112	70	444	o
J. R.	o	0.75	6.7	70/179	90	455	o
A. B.	o	0.75	7.6	54/154	78	*265*	o

PATIENTS WITH COMBINED LESIONS

Patient	Hepatomegaly	Serum bilirubin mgm./100 ml.	Serum protein G/100 ml.	Cholesterol /esters mgm./100 ml.	MCV μ^3	Glucuronates mgm./day	Patient considered to have abnormal degree of hepatic insufficiency
A. S.	o	*1.10*	7.8	64/135	81	503	o
D. B.	o	0.45	7.1	....	90	360	o
B. B.	..	0.87	..	70/171	82	363	o
A. B.	o	0.50	7.0	....	80	...	o
A. C.	o	0.90	7.2	60/147	*100*	283	o
C. B.	+	0.63	7.5	*49/93*	79	600	o
D. M.	o	...	..	....	90	175	o
A. K.	o	0.75	7.6	69/178	83	448	o
F. W.	+	*1.40*	7.0	55/118	92	*234*	+
P. K.	+	*1.25*	6.5	67/208	91	820	+
C. K.	+	*1.10*	7.7	*76/104*	80	*100*	+
L. D.	+	0.80	7.1	72/144	85	*91*	o
B. H.	o	*1.00*	6.9	....	89	340	o
T. R.	o	0.45	8.5	79/181	81	582	o
A. B.	o	0.90	7.1	50/123	90	...	o
S. J.	o	0.70	7.3	....	*96*	537	o
W. K.	+	0.50	7.4	42/191	90	*236*	o
E. McG.	o	0.25	7.8	43/107	80	712	o
P. N.	o	0.63	7.4	....	81	...	o
J. S.	o	...	..	70/146	80	594	o
D. S.	o	0.70	7.8	*54/105*	74	...	o

* Values in italic are indicative of abnormal hepatic function.

TABLE IV: THE INCIDENCE OF GASTROINTESTINAL SYMPTOMATOLOGY IN THE PATIENTS STUDIED

Patient	Sore mouth or glossodynia	Dysphagia	Epigastric pain	Constipation	Weight loss	Gastric achlorhydria	Gastroscopic examination	X-ray of esophagus	X-ray of stomach and intestines
PATIENTS WITH GLOSSAL PAPILLARY ATROPHY									
A. W.	+	o	..	..	o	+	Negative	Negative	Negative
L. O.	+	+	+	..	o	+	Negative	Spasm	Hypermotility
H. K.	o	o	..	..	+	o	Negative	Negative	Negative
S. U.	+	+	+	+	+	+	Mucosal atrophy	Spasm	Negative
M. R.	+	+	+	+	+	o	Negative	Negative	Mucosal atrophy
H. J.	+	+	o	o	o	o	Mucosal atrophy	Spasm	Negative
G. O.	o	+	o	o	+	+	.....	Spasm	Hypermotility
C. F.	+	o	o	o	o	o	Negative	Negative	Negative
G. M.	+	+	+	o	o	o	Negative	Negative	Duodenal diverticulum
C. B.	+	o	+	+	o	o	Negative	Negative	Hypermotility
A. D.	+	o	o	o	o	o	Mucosal atrophy	Negative	Mucosal atrophy
I. R.	o	+	o	+	+	+	Mucosal atrophy	Negative	Negative
G. K.	o	o	+	..	o	o	Mucosal atrophy	Negative	Negative
H. F.	+	o	..	o	o	o	Mucosal atrophy	Spasm	Negative
S. J.	o	o	..	..	o	o	Negative	Negative	Hypermotility
G. C.	o	o	o	..	+	+	.....	Negative	Negative
I. L.	+	o	+	o	o	+	.....	Traction diverticulum	Hiatus hernia
PATIENTS WITH ORAL LEUKOPLAKIA									
M. J.	+	o	+	+	o	o	Negative	Negative	Negative
D. L. B.	+	+	o	+	o	o	Negative	Hiatus hernia	Negative
D. L.	+	+	+	+	+	+	Negative	Diverticulum	Duodenal diverticulum
H. G.	..	..	..	..	..	+	.....	Negative	Negative
J. B.	+	o	o	o	o	o	Negative	Negative	Negative
I. S.*	+	+	+	+	o	+	Negative	Negative	Rectal leukoplakia
E. S.	o	o	o	o	+	o	Negative	Negative	Duodenal ulcer
J. B.	+	o	o	o	o	o	Negative	Negative	Hiatus hernia
E. P.	+	o	o	o	+	..	.....	.....	Negative
E. K.	+	o	..	..	o	o	Negative	Negative	Negative
S. K.	o	o	..	..	o	+	Negative	Spasm	Negative
J. M.	o	o	..	..	o	..	Negative	Negative	Negative
I. C.	+	o	..	..	o	o	.....	.....	.....
I. M.	o	o	..	..	o	..	.....	.....	.....
H. R.	+	o	+	+	o	o	.....	Negative	Negative
J. D.	o	o	o	o	o	o	.....	Spasm	Negative
F. F.	o	o	..	..	..	o	Negative	Diverticulum	Negative
L. F.	o	o	..	..	+	o	Negative	Negative	Negative
H. J.	o	o	..	..	o	+	.....	Negative	Negative
J. W.	o	o	+	o	o	..	Negative	Negative	Negative
J. N.	o	o	o	o	o	o	.....	Negative	Negative
E. N.	+	o	+	o	+	+	Mucosal atrophy	Negative	Duodenal ulcer, pylorospasm
H. S.	o	o	o	+	o	o	Negative	Negative	Negative
E. R.	+	o	o	o	+	o	Negative	Negative	Negative
S. S.	+	+	o	o	..	o	.....	Negative	Negative
G. S.	+	o	o	o	o	o	Negative	Diverticulum	Negative
G. Y.	o	o	o	o	o	o	Negative	Negative	Hiatus hernia
J. R.	o	o	+	o	o	o	Negative	Negative	Negative
A. B.	+	o	o	o	o	o	.....	Negative	Negative
PATIENTS WITH COMBINED LESIONS									
A. S.	+	o	+	+	+	o	Mucosal atrophy	Negative	Negative
D. B.	+	+	+	+	+	o	Mucosal atrophy	Negative	Mucosal atrophy
B. B.	+	o	..	+	+	o	Negative	Negative	Hiatus hernia
A. B.	o	+	..	..	+	+	Negative	Negative	Negative
A. C.	+	o	..	+	+	+	Negative	Negative	Negative
C. B.	+	+	+	o	+	o	Negative	Negative	Negative
M. M.	o	o	o	o	o	o	Negative	Negative	Negative
A. K.	+	o	o	+	o	o	.....	Negative	Negative
F. W.	+	+	+	+	+	o	.....	Mucosal atrophy	Mucosal atrophy
S. K.*	o	o	..	..	o	o	Negative	Negative	Negative
B. K.	o	o	o	..	o	o	.....	.....	.....
L. D.	+	o	..	+	o	o	.....	Negative	Functional changes
B. H.	o	o	..	+	o	+	Negative	Negative	Negative
T. R.	+	o	..	..	o	+	Negative	Negative	Pylorospasm
E. B.	o	o	..	..	o	+	Negative	Negative	Negative
S. J.	o	o	o	..	o	o	Negative	Negative	Negative
E. K.	+	o	o	o	+	o	Negative	Negative	Negative
E. McG.	o	o	+	o	o	+	.....	Negative	Negative
P. N.	+	o	+	o	o	o	Negative	Negative	Negative
G. S.	+	o	o	o	+	o	Negative	Negative	Negative
S. S.	+	+	+	o	o	o	Mucosal atrophy	Negative	Mucosal atrophy

* Rectal leukoplakia.

cent) were constipated for from 2 to 20 years. Histories of gradual but progressive weight loss (from 3 to 20 pounds) were obtained in 7 instances (41 per cent).

Of the patients with oral leukoplakia, 4 (14 per cent) suffered from dysphagia, and 7 (24 per cent) from postprandial epigastric pain. Seven others (24 per cent) had progressive constipation, and 5 (17 per cent) had lost weight. These same symptoms were encountered more frequently in the patients with the combined lesions, of whom 5 (21 per cent) had dysphagia, 7 (33 per cent) epigastric pain, 8 (38 per cent) had constipation and 9 (43 per cent) had a significant weight loss.

It is interesting to note that the incidence of symptoms referable to the gastrointestinal tract was more common in the two groups of patients with papillary atrophy of the tongue than in the group of individuals with only oral leukoplakia.

Functional and organic changes in the gastrointestinal tracts of the patients studied frequently were found by x-ray examination and gastroscopy (Table IV). These abnormalities were most common in the 17 patients with papillary atrophy of the tongue. Gastroscopic examination was performed in 14 patients and revealed atrophy of the gastric muscosa in 6 (43 per cent). In one instance this observation was confirmed by x-ray. X-ray examination further showed that 5 other patients of the group had functional changes of the esophagus and 6 had similar changes of the stomach; 1 had a traction diverticulum, 1 had a hiatus hernia of the esophagus, and 1 patient had a diverticulum of the duodenum. Thus, of the 17 patients, 13 (77 per cent) had either x-ray or endoscopic evidence of pathologic changes in the gastrointestinal tract.

Similar, but less frequent, pathologic changes of the gastrointestinal tract were observed in the 29 patients with oral leukoplakia. Three had esophageal spasms, 3 had esophageal diverticula, 3 had hiatus hernias of the stomach, 2 had duodenal diverticula, and 1 had atrophy of the gastric mucosa. One patient was found to have a marked spastic colitis, another had extensive polyposis of the sigmoid, and a third had recto-anal leukoplakia. Thus 13 (45 per cent) had either x-ray or endoscopic evidence of organic or functional change of the gastrointestinal tract.

Finally, an investigation for similar changes in 20 of the 21 patients with the combined lesions revealed that 4 had atrophy of the gastric mucosa, 2 had pylorospasm, 1 had a hiatus hernia, and 1 had esophageal stenosis. Thus, 8 (40 per cent) had functional or anatomic gastrointestinal pathology. This incidence of gastrointestinal pathology found in the patients studied would appear to indicate that their oral or lingual lesions were related to a generally deranged physiology of the alimentary tract.

b. *Cheiloses and perleche.*—Since this investigation was concerned particularly with the existence of dietary deficiency in the patients studied, the accepted signs of vitamin deficiency were sought carefully. Of the numerous signs, perleche and cheiloses were encountered most frequently.

Well-defined perleche and cheiloses were observed in 9 (53 per cent) of the 17 patients with glossal papillary atrophy, in 5 (17 per cent) of those with oral leukoplakia, and in 8 (37 per cent) of 21 patients with the combined lesions. However, it is interesting to note that of these 20 patients with lesions considered to be due to a riboflavin deficiency (19), only 3 had abnormally low urinary excretions of that vitamin. Eight other individuals who did have low urinary outputs of riboflavin had neither cheiloses nor perleche.

c. *Nails.*—The nails of the patients investigated frequently were found to be abnormal. These abnormal nails were exceptionally thick and longitudinally striated, markedly friable, and had frayed and tender cuticles. Nails of this type were found in 10 of the 17 patients with papillary atrophy of the tongue, and in 4 of these individuals the nails also were spoon-shaped.

Likewise, these nail abnormalities were noted in 10 of the 29 patients with oral leukoplakia, and in 9 of the 21 patients with the combined lesions.

It is impossible to state at this time the cause of such abnormal nails. The association of koilonychia with persistent microcytic anemia is well known, but in the present instance that explanation as a cause for the incidence of nail abnormalities is not tenable. Of all the 29 patients with abnormal nails, only 4 had microcytic anemia. The possibility that their nail deformities were related to a deficient ingestion of sulfur amino acids is a more likely explanation, for of the 29 patients, 20 gave dietary histories which were deficient in protein.

d. *Anemia.*—Despite the fact that apparently so many of the patients studied had gastric anacidity or had been on deficient diets, the incidence of anemia among them was not correspondingly great. Patients were considered anemic when their red cell counts were less than 4 million, and hemoglobin less than 80 per cent. By these standards anemia was found in only 7 of the 17 patients with papillary atrophy of the tongue, in 3 of the 29 with oral leukoplakia, and in 6 of the 21 with the combined lesions. Of the 16, the anemia in 6 individuals was microcytic and all had been on grossly inadequate diets. Two individuals had macrocytosis, but neither had any gastric mucosal atrophy nor a significant degree of hepatic insuffi-

ciency. The anemia of the remaining 8 patients was normocytic and normochromic.

In summary, the pathologic changes most frequently associated with glossal papillary atrophy and oral leukoplakia are gastric achlorhydria; functional and organic changes in the gastrointestinal tract; cheiloses and perleche; thick, striated nails; and occasionally varying degrees and types of anemia. There is no evidence at present to indicate whether all of these are coincidental findings or are related in any etiologic way to the oral lesions.

3. The Effect of the Administration of Large Supplements of Yeast to Patients with Papillary Atrophy of the Tongue and Oral Leukoplakia

The evidence thus far obtained in this investigation has indicated, in the patients studied, an intimate relationship between the existence of a simple or conditioned dietary deficiency and the occurrence of glossal papillary atrophy or oral leukoplakia. This evidence was based upon the observations (a) that from 55 to 61 per cent of the patients for at least 3 years had been on diets which contained inadequate amounts of cereals, fruits, fresh or cooked vegetables, and animal proteins, (b) that 42 per cent of the patients had low plasma levels of vitamin A, low urinary excretions of riboflavin, or both, (c) that 30 per cent of the patients had gastric achlorhydria, and (d) that 12 per cent had significant degrees of hepatic dysfunction which conceivably interfered with the proper utilization of absorbed essential dietary factors.

To test further the relationship between the inadequate ingestion of protein and vitamin B complex, and the occurrence of papillary atrophy of the tongue and oral leukoplakia, 54 of the 67 patients included in this investigation were given daily from 60 to 120 gm. of brewer's yeast[1] for from 2 to 10 months. The patients who had gastric achlorhydria also received daily 30 minims of U.S.P. dilute hydrochloric acid, and those who were anemic were given 30 grains of ferrous sulfate each day. During the periods of treatment the patients usually were observed once every 2 weeks.

Of the 17 patients with papillary atrophy of the tongue, yeast and, when necessary, hydrochloric acid and iron, were administered to 16. In 7 instances (44 per cent) marked subjective and objective improvement was observed in from 2 to 10 months. In

[1] The yeast used in this investigation was Fleischmann's 20-40 granular brewer's yeast. This material contains per gram 50 I.U. of thiamin, 70 μgm. of riboflavin, 450 μgm. of nicotinic acid, 6 μgm. of pyridoxin, 150 μgm. of pantothenic acid, and 524 mgm. of protein; also significant amounts of sulfur amino acids.

Table V: The Results of the Administration of Yeast to the Patients Studied

Patient	Moderate improvement		Marked improvement	
	Subjective	Objective	Subjective	Objective
PATIENTS WITH GLOSSAL PAPILLARY ATROPHY				
A. W.	..	..	+	+
L. O.	..	..	+	+
H. K.	o	o	..	..
S. U.	+	+	..	..
M. R.	..	..	+	+
G. J.	+	+	..	..
C. F.	+	+	..	..
G. M.	o	o	..	..
C. B.	+	+	..	..
A. D.	o	o	..	..
I. R.	..	..	+	+
G. K.	..	..	+	+
J. F.	..	..	+	+
C. J.	+	+	..	..
T. C.	..	..	+	+
I. L.	+	+	..	..
PATIENTS WITH ORAL LEUKOPLAKIA				
A. J.	..	..	+	+
D. L. B.	o	o	..	..
D. L.	o	o	..	..
H. C.	+	+	..	..
E. S.	o	o	..	..
J. B.	+	+	..	..
A. K.	+	+	..	..
H. K.	o	o	..	..
M. M.	o	o	..	..
I. C.	+	+	..	..
I. M.	+	+	..	..
J. R.	+	o	..	..
J. F.	+	+	..	..
L. F.	o	o	..	..
W. J.	o	o	..	..
J. W.	..	..	+	+
F. N.	+	+	..	..
W. N.	+	o	..	..
J. S.	+	o	..	..
W. R.	+	o	..	..
J. S.	..	..	+	+
G. Y.	..	..	+	+
J. R.	o	o	..	..
PATIENTS WITH COMBINED LESIONS				
A. S.	..	..	+	+
D. B.	o	o	..	..
B. B.	o	o	..	..
A. C.	..	..	+	+
C. B.	o	o	..	..
D. M.	+	+	..	..
P. K.	+	+	..	..
C. K.	o	o	..	..
L. D.	o	o	..	..
B. H.	+	+	..	..
T. R.	+	+	..	..
A. B.	+	+	..	..
S. J.	o	o	..	..
W. K.	+	+	..	..
D. S.	+	+	..	..

those 7 the glossodynia and dysphagia completely remitted, and a fine regrowth of the glossal papillae occurred. Similar, but lesser degrees of improvement were noted also in 6 other instances (37 per cent).

In 3 patients (G. M., H. K., A. D.), however, the administration of yeast did not effect any remission of either the symptoms or the lingual lesions, but of these 3 patients 2 previously had not been on inadequate diets, and 1 of those 2 had a significant degree of hepatic dysfunction.

Of the 29 patients with oral leukoplakia, 23 were treated either with yeast alone, or with yeast, iron, and hydrochloric acid. Although the results obtained in this group apparently were not as successful as were those in the patients with glossal papillary atrophy, beneficial effects undoubtedly were obtained in several instances by the administration of the yeast. In 4 patients (17 per cent) all or a large part of the leukoplakic lesion disappeared, leaving in its site a thin patch of mucous membrane atrophy. In those 4, all the local symptoms likewise remitted. In 7 (30 per cent) other patients the leukoplakic lesions became thinner and lost some of their yellow-white appearance. Fifteen (55 per cent) of the patients, while on yeast therapy, repeatedly affirmed their subjective improvement; those who previously had glossodynia or dysphagia no longer complained of those symptoms.

Eight of the 23 individuals showed neither subjective nor objective improvement after from 2 to 10 months of continual therapy. Again of these 8, 3 previously had taken normal diets, and 1 other had an abnormal degree of hepatic insufficiency which might have accounted for an inadequate utilization of absorbed dietary constituents.

Finally, of the 21 patients with combined lesions, 15 were treated by the administration of yeast, and

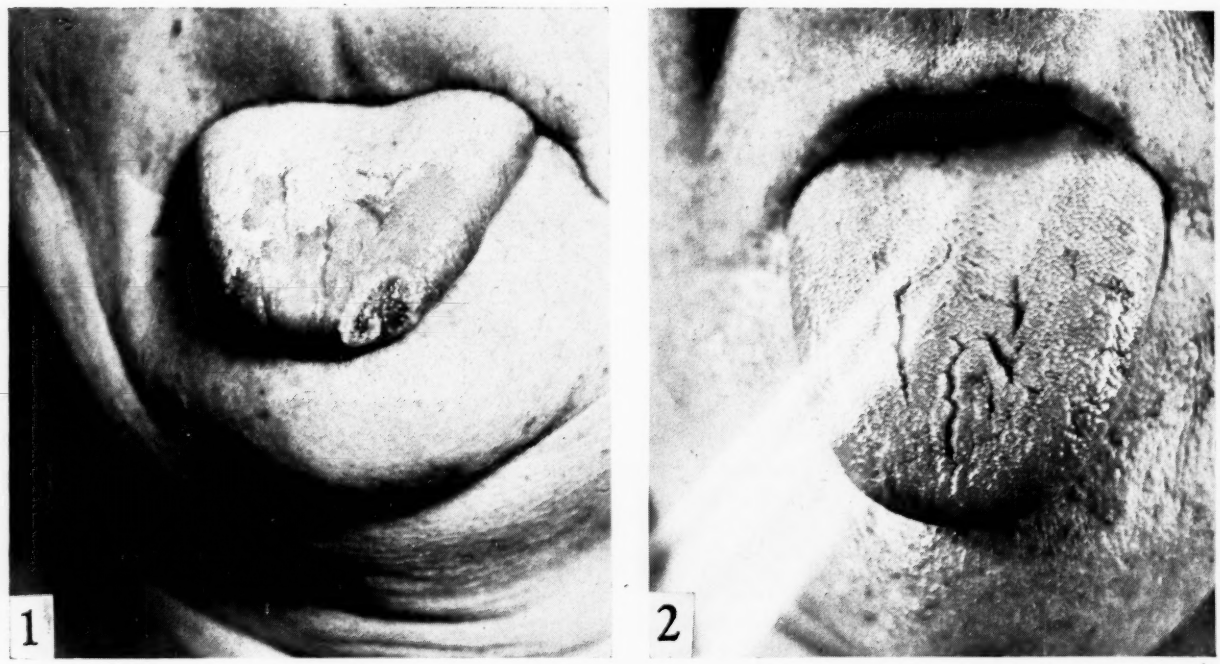


FIG. 1.—Patient A. W., showing patches of papillary atrophy of the tongue. Yeast therapy started on January 21, 1939.
FIG. 2.—Patient A. W., July 11, 1939. After 6 months of yeast therapy there has been regrowth of glossal papillae. At this time yeast therapy was discontinued.

DESCRIPTION OF FIGURES 3 TO 8

FIG. 3.—Patient A. J., showing leukoplakic patch on the left dorsal aspect of the tongue. Yeast therapy started on April 18, 1940.

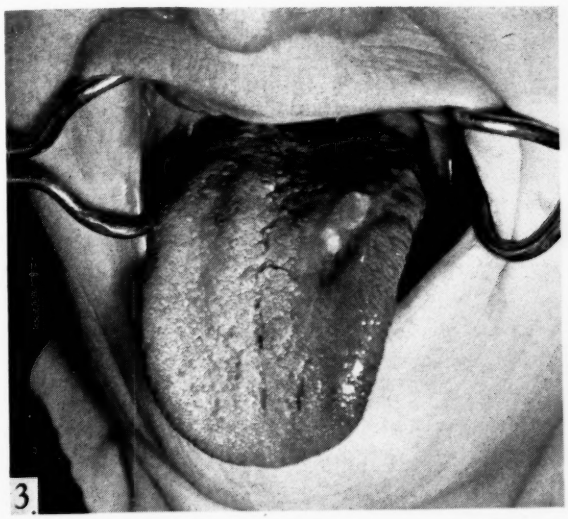
FIG. 4.—Patient A. J., October 17, 1940. Almost complete disappearance of the lesion after 6 months of yeast therapy. Treatment was discontinued.

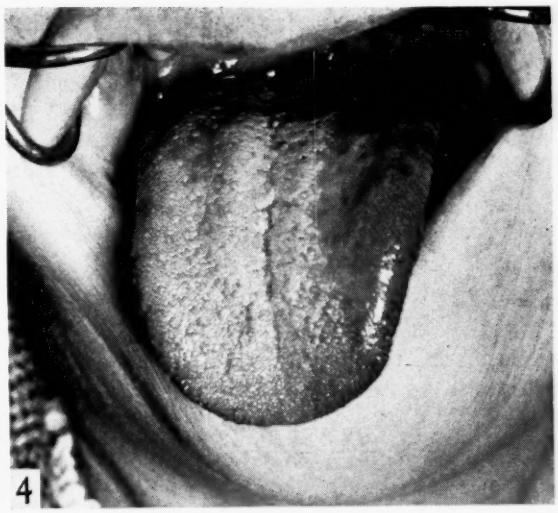
FIG. 5.—Patient A. J., December 12, 1940. After the patient had discontinued yeast therapy for 2 months the leukoplakic lesion on the left dorsal aspect of the tongue returned. Yeast therapy reinstituted.

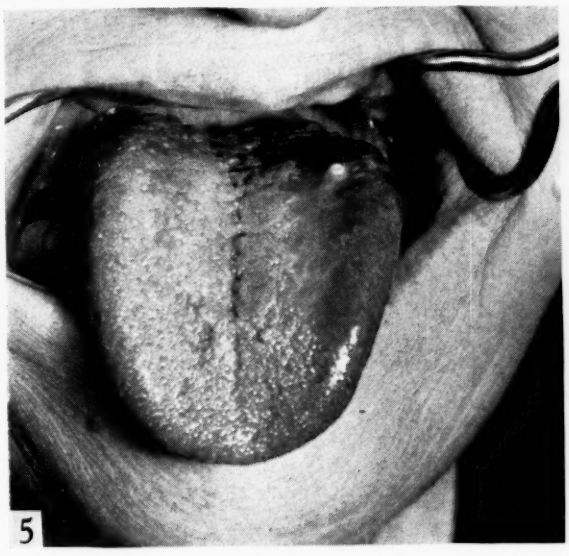
FIG. 6.—Patient A. J., May 15, 1941. After 6 months of yeast therapy the leukoplakic lesion of the left dorsal aspect of the tongue has disappeared.

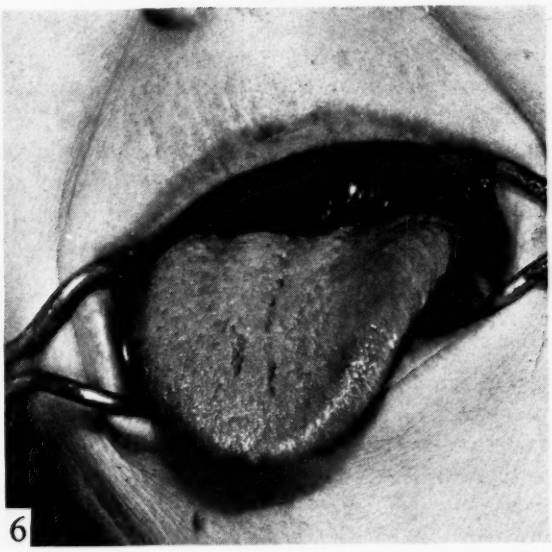
FIG. 7.—Patient C. G., showing leukoplakia of the gingiva, inner surface of the labial commissure, lip, and buccal mucosa. Yeast therapy started January 17, 1941.

FIG. 8.—Patient C. G., October 21, 1941. After 9 months of yeast therapy there has been marked remission in the extent and thickness of the leukoplakic process.









FIGS. 3-8

occasionally by dilute hydrochloric acid and iron. Marked objective and subjective improvement after from 2 to 10 months of this therapy were noted only in 2 individuals (13 per cent). In both, these responses consisted chiefly of a regrowth of fine papillae of the tongue and a disappearance of the thin buccal leukoplakia. In 7 other patients (47 per cent), some decrease in the thickness and extent of the leukoplakic lesions was noted (especially those lesions of the tongue), and all of these 7 repeatedly admitted considerable relief from their previous glossodynia, sore mouths, or dysphagia.

Six patients of this group failed to obtain either subjective or objective improvement from the treatment. Two of these 6 previously had been on adequate diets, and 1 other had a considerable degree of hepatic dysfunction.

In summary, it would appear that the administration of large quantities of yeast to patients with papillary atrophy of the tongue or oral leukoplakia was followed occasionally by a complete or by a partial remission of their lesions and oral symptoms. This observation would indicate the existence of a close relationship between the appearance of such lesions and a lack of certain dietary factors present in yeast.

DISCUSSION

The evidence presented has indicated that the majority of patients with papillary atrophy of the tongue probably suffered from dietary deficiency. In most instances that deficiency existed simply because the diets taken were poor in meats, fish, cereals, fruits, and vegetables. In other patients, because of their hepatic dysfunction, gastric achlorhydria, anatomic lesions and functional alterations of the gastrointestinal tract, it was reasonable to assume that a malabsorption or defective utilization of essential dietary constituents existed. Likewise, the symptoms of glossodynia and sore mouth, of which 7 of the 17 patients complained, probably restricted further in those patients a desire to ingest or masticate food and, therefore, must be regarded as an additional cause of the dietary deficiency. The observations that 6 of the 17 patients had chemical evidence of vitamin A or B_2 deficiency, and that 9 had cheiloses and perleche, lesions due to a lack of vitamin B complex, substantiated the existence of dietary deficiency in this group of individuals.

Although it was possible to discover 1 or more reasons for the existence of dietary deficiency in 80 per cent of the patients with papillary atrophy of the tongue, it nevertheless was necessary to obtain more direct proof that the dietary deficiency had induced the glossal lesions. This proof was provided by the observation that the administration of brewer's yeast to 16 of these patients was followed by both subjective and objective improvement in all but 1 instance. Long-continued deprivation of vitamin B complex or nicotinic acid (21) from the diet has been known to produce in human beings atrophy of the glossal papillae.

It is impossible at this time to state whether or not the functional and organic changes in the gastrointestinal tract of these patients also were secondary to their dietary deficiency. There are numerous observations which strongly suggest that changes in the motility of the gastrointestinal tract (21) can be induced by an inadequate ingestion of vitamin B complex. There are also some observations which show that atrophy of the gastric mucosa, a lesion found in 43 per cent of the patients studied who had glossal papillary atrophy, probably is related to an insufficient intake of factors present in liver extract (17). Finally, diverticula of the esophagus, stomach, or duodenum frequently are associated with disturbances in absorption from the alimentary canal (6, 10, 15). Thus it is obvious that, if dietary deficiency were the cause for these lesions which further aggravated that deficiency, a vicious cycle would be set up.

Although the relationship between dietary deficiency and oral leukoplakia is not as clear as that between dietary deficiency and glossal papillary atrophy, nevertheless, the evidence at hand would indicate that such a relationship does exist. Reasons for a simple or conditioned dietary deficiency have been demonstrated in 61 per cent of the patients studied. Again, the administration of yeast to 23 of the 29 patients with oral leukoplakia produced both subjective and objective improvement in 15. In 4 that improvement was considered to be marked.

If it is accepted that papillary atrophy of the tongue in the patients studied was due to a dietary deficiency of certain factors present in yeast, then the association of that lesion with oral leukoplakia in 46 of 67 patients (70 per cent) would suggest that oral leukoplakia likewise had been induced by the deficient state. This suggestion is supported further by the occurrence of cheiloses and perleche in 5 of the 29 patients with oral leukoplakia alone, and in 8 of the 21 patients with both oral leukoplakia and glossal papillary atrophy.

The mechanism by which a deficient intake of vitamin B complex results in atrophy or keratinization of epithelial structures is not clear. Recent studies have shown that the dermal layers of riboflavin-deficient rats have a markedly decreased respiratory activity (2). Similarly, the tissues of animals on an inadequate intake of vitamin B complex (9) also have a decreased utilization of oxygen. Although direct measurements of the respiration of oral and glossal epithelium have not been made, it is con-

ceivable that the respiratory activity of those tissues, too, might be decreased in the vitamin B complex-deficient animal.

It is difficult to evaluate as yet the importance of the excessive use of tobacco by the patients studied. Tobacco fumes probably act as an irritant and the irritation of an already physiologically deranged epithelium might result in the death of that tissue. Why in some instances the epithelium merely should atrophy and in others produce a thick layer of leukoplakia cannot be answered at this time.

SUMMARY AND CONCLUSIONS

1. The incidence of dietary deficiency and the excessive use of tobacco were found to be high in patients with papillary atrophy of the tongue, with oral leukoplakia, and with combinations of those lesions. The incidence of syphilis in these patients was not abnormally great.

2. In many patients studied the incidence of simple dietary deficiencies was confirmed by the finding of low plasma levels of vitamin A and low urinary excretions of riboflavin.

3. The pathological changes associated with papillary atrophy of the tongue and oral leukoplakia in the patients studied were hepatic dysfunction, anatomical and functional abnormalities of the gastrointestinal tract, anemia, nail deformities, cheiloses, and perleche. Hepatic dysfunction and abnormalities of the gastrointestinal tract were considered to be factors which might contribute to the existence of a dietary deficiency.

4. The administration of large quantities of brewer's yeast to patients with papillary atrophy of the tongue or oral leukoplakia was followed occasionally by a complete or partial remission of their lesions and oral symptoms. This observation suggests the existence of a relationship between the appearance of such lesions and a lack of certain dietary factors present in yeast.

REFERENCES

1. Abels, J. C., Rekers, P. E., Binkley, G., Pack, G. T., and Rhoads, C. P. Metabolic Studies in Patients with Gastro-Intestinal Cancer. II. Hepatic Dysfunction. Ann. Int. Med., **16**:221-240. 1942.
2. Adams, P. D. The Oxygen Uptake and Composition of Skin of Rats in Vitamin G Deficiency. J. Biol. Chem., **116**:641-651. 1936.
3. Bloomfield, A. L., and Poland, W. S. Gastric Anacidity, Its Relation to Disease. P. 178. New York: Macmillan Company. 1933.
4. Cates, H. B. Relation of Liver Function to Cirrhosis of the Liver and Alcoholism: Comparison of Results of Liver Function Tests with Degree of Organic Change in Cirrhosis of Liver and with Results of Such Tests in Persons with Alcoholism without Cirrhosis of Liver. Arch. Int. Med., **67**:383-398. 1941.
5. Clausen, S. W., and McCoord, A. B. Carotenoids and Vitamin of the Blood. J. Pediat., **13**:635-650. 1938.
6. Clerf, L. H. Diseases of the Oesophagus. Arch. Surg., **41**:1043-1059. 1940.
7. Cutler, M., and Buschke, F. Cancer, Its Diagnosis and Treatment. P. 104. Philadelphia: W. B. Saunders Company. 1938.
8. Ferrebee, J. W. Urinary Excretion of Riboflavin: Fluorometric Method for Its Estimation. J. Clin. Invest., **19**: 251-256. 1940.
9 Hastings, A. B., Muus, J., and Bessey, O. A. Tissue Metabolism in Vitamin Deficiencies. I. Effect of Deficiencies of Riboflavin and Other Heat-Stable Vitamin B Components. J. Biol. Chem., **129**:295-301. 1939.
10. Jordan, S. M., and Lahey, F. H. Diverticula of the Alimentary Tract. Surg. Clin. N. Amer., **6**:747-765. 1926.
11. Landor, J. V., and Pallister, R. A. Avitaminosis B_2. Tr. Roy. Soc. Trop. Med. & Hyg., **29**:121-134. 1935.
12. MacIntyre, D. S., Pederson, S., and Maddock, W. G. Glycogen Content of Human Liver. Proc. Soc. Exper. Biol. & Med., **47**:354-355. 1941.
13. Manson-Bahr, P. Glossitis and Vitamin B_2 Complex in Pellagra, Sprue, and Allied States. Lancet, **2**:356-359. 1940.
14. Maugham, A. B., Evelyn, K. A., and Browne, J. S. L. A Method for Quantitative Estimation of Glucuronic Acid and Conjugated Glucuronides. J. Biol. Chem., **126**:567-572. 1938.
15. Mayo, C. Diverticula of the Gastro-Intestinal Tract; Their Surgical Importance. J. A. M. A., **59**:260-264. 1912.
16. Musser, J. H., and Wintrobe, M. M. In Tice, F. System of Medicine. Vol. 6, pp. 795-797. Hagerstown, Md.: W. F. Prior. 1940.
17. Schindler, R., Kirsner, J. B., and Palmer, W. L. Atrophic Gastritis. Gastroscopic Studies on Effects of Liver and Iron Therapy: Preliminary Report. Arch. Int. Med., **65**:78-89. 1940.
18. Schoenheimer, R., and Sperry, W. M. Micromethod for the Determination of Free and Combined Cholesterol. J. Biol. Chem., **106**:745-760. 1934.
19. Sebrell, W. H., and Butler, R. E. Riboflavin Deficiency in Man. Pub. Health Rep., **54**:2121-2131. 1939.
20. Sofer, L. J., and Paulson, M. Comparative Advantage and Further Modification of the Bilirubin Excretion Test for Hepatic Function. Am. J. M. Sc., **192**:535-540. 1936.
21. Spies, T. D., Vilter, R. W., and Ashe, W. F. Pellagra, Beriberi and Riboflavin Deficiency in Human Beings: Diagnosis and Treatment. J. A. M. A., **113**:931-936. 1939.
22. Todd, J. C., and Sanford, A. H. Clinical Diagnosis by Laboratory Methods. Eighth edition. P. 403. Philadelphia: W. B. Saunders Company. 1936.
23. Weech, A. A., Reeves, E. B., and Goettsch, E. The Relationship Between the Specific Gravity and Protein Content in Plasma, Serum and Transudate from Dogs. J. Biol. Chem., **113**:167-174. 1936.

Mastopathia Cystica and Mammary Carcinoma

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The relationship of cystic disease of the breast, mastopathia cystica, to carcinoma is, and has always been, a subject of controversy. In 1906, when Bloodgood (2) considered senile parenchymatous hypertrophy, he and many other authors thought that this condition was a precancerous lesion and that the probability of cancer being associated with it was as high as 50 per cent. However, in 1921 (3) he described a group of 350 patients with chronic cystic mastitis, in 222 of whom he had had the opportunity to study the entire breast. In not a single instance did he find gross or microscopic cancer. In 1932 (4), a check-up examination on these patients showed that none had developed evidence of malignancy. At this time he added 30 new operative cases and almost 200 examples of unoperated "shotty" breast which had been followed by yearly examination. He reaffirmed the opinion formulated 10 years previously that the development of cancer of the breast after removal of a zone of the breast, which is the seat of chronic cystic mastitis, is apparently not more frequent than in the same number of women at the same age upon whom operation has not been performed (3).

Deaver and McFarland (10), after examining 335 cancerous breasts, found 23 in which abnormal involution and carcinoma coexisted, but stated that they "cannot see the necessity for supposing that two conditions that happen to coexist must be connected to one another in the relation of cause and effect."

Kilgore (17) found carcinoma in only 1 of 19 instances of hyperplastic cystic disease showing clinically as a lump in the breast. Klingenstein (18) presented the follow-up data on 54 of an original group of 74 patients who had had partial excision of a breast for chronic cystic mastitis. Only 2 patients (3.7 per cent) were known to have developed carcinoma. Campbell (5) reported 43 examples of hyperplastic cystic disease among 233 cases of cystic disease of the breast of all types, which had been treated by local excision. In only one did carcinoma of the breast subsequently develop and then in an area far removed from the site of the previous operation. Of 57 patients treated by amputation of one breast, carcinoma developed in the remaining breast of but one. Thus, of 290 patients followed, only 2 or 0.7 per cent developed carcinoma. Johnson (15) followed 61 of 101 patients with cyst of the breast for periods of 1 to 20 years and found no evidence of carcinoma. In 4 instances in which both a cyst and carcinoma were dealt with, the carcinoma was on the same side as the cyst in 2 and on the opposite side in 2.

Lewis and Geschickter (19) also emphasized Bloodgood's views. They reported 3 deaths from carcinoma among 271 patients with adenosis of the breast who had been followed for more than 5 years, and 1 death from carcinoma among 252 patients with cystic disease followed for the same period. In a study of 2,675 carcinomas of the breast, only 0.5 per cent of the usual types were found to show changes characteristic of Schimmelbusch's disease, yet 30 per cent having carcinoma of the ductal (comedo) type were associated with breast tissue showing these changes. Greenough and Simmons (14) traced 83 women with partial resection of the breast for cystic disease over periods of 1 to 17 years (average 7 years). In 4 (4.8 per cent) carcinoma developed in the remaining breast tissue. Fischer (13) found cystic disease in 14 per cent of 151 patients. Rodman (22) found that chronic cystic mastitis was associated with carcinoma in 15.5 per cent of more than a hundred cases studied by him. Cheatle and Cutler (7, 8) stated that at least 20 per cent of all carcinomas of the breast can be traced directly to mastopathia cystica. This figure is in close agreement with that of Semb (23), who found the incidence of carcinoma in 100 cases of "fibro-adenomatosis cystica" to be 24 per cent, and of Morpurgo (21), who found cystic changes in 24 per cent of 196 patients with carcinoma of the breast. Dietrich and Frangenheim (11) studied 500 mammary gland preparations of which 233 (46.6 per cent) showed mastopathia cystica. Sixty-eight of the 233 (29.2 per cent) showed coexistent carcinoma.

Ewing (12) found minimal carcinomas in 50 per cent of the breasts excised for cystic disease. Dahl-Iverson and Starup (9) found coincidence of carcinoma and mastopathia cystica in 44 per cent of 34 cases studied by them. Keynes (16) reported on 116 specimens of breast tissue removed post-mortem, of which 57 were normal and 59 showed "inflammatory lesions." He also reported that in 25 examples of carcinoma of the breast, 20 (80 per cent) showed some evidence of "chronic mastitis." Upon histologic study, Charteris (6) found that 41 (85 per cent) of 48 breasts removed for carcinoma were also the seat of chronic cystic mastitis in some degree. The malignant growth was believed to have arisen from the ducts in 31 of the 48 (64 per cent). MacCarty and Mensing (20) examined 967 breasts removed for carcinoma and found evidence of mastopathia cystica in 100 per cent of them.

Thus, it can be seen that a historical review of the literature from either a clinical or pathologic viewpoint will reveal considerable evidence to support either the casual or the causal relationship of mastopathia cystica and carcinoma. The most recent report is that of Warren (24), who followed clinically for at least 5 years a group of women who had portions of the breast removed. He compared the incidence of carcinoma in this group with that of the general female population and found that 35 cancers occurred in 1,044 individuals with chronic mastitis and chronic cystic mastitis. On contrasting specific cancer rates with those of the female population of Massachusetts, evidence was found that the cancer rate for women with pre-existing breast lesions is 4.5 times as great as for all women, and that this predominance is especially marked in the decades below 50 years of age.

The definition of mastopathia cystica depends, to a large degree, upon each author's opinion as to the nature of the disease. Cheatle and Cutler (7) considered that mastopathia cystica includes two distinct diseases. The first, named "mazoplasia," is a physiologic rather than a pathologic process, and there are no other lesions in the breast such as fibroadenomas, cysts, or papillomas. It is characterized by desquamation of the epithelial cells in the terminal ducts and acini, accompanied by hyperplasia of the pericanalicular and periacinous connective tissue. The second type of epithelial hyperplasia results in the formation of cysts, minute to large, and is designated "cystipherous desquamative epithelial hyperplasia." It was the opinion

of these authors that this type of hyperplasia terminates in neoplasia in at least 20 per cent of the cases.

Whitehouse (25) pointed out that in his investigations he never saw cystipherous hyperplasia in a breast which did not, in some part, show also mazoplasia. He felt that mazoplasia was apparently the natural result of excessive stimulation of the mammary epithelium by the luteal hormone, and that repeated cyclical stimulation of the mammary epithelium results in the production of excessive secretion and cystic disease. He suggested the term "mastopathia" to designate that stagnation of the mammary gland which results from the lack of physiologic monthly drainage of that organ.

Willer (26) and Berning and Bücker (1) considered the apocrine glands very important in the formation of benign and malignant neoplasms. Willer found that apocrine glands are rare in the normal breast, except in the periphery, but that they occur with great regularity in mastopathia cystica. Berning and Bücker made the diagnosis of mastopathia cystica upon the presence of pale-celled epithelium with eosinophilic granules. The name "pale epithelium" is derived from the fact that in such epithelial cells the nucleus is very small in comparison to the size of the cell and lies in its basal part. They also described epithelial proliferation with formation of true and pseudo-growths but without division figures or infiltration.

The Morphologic Complex of Mastopathia Cystica

Our concept of the morphology of mastopathia cystica includes the presence of dilated ducts accompanied by diffuse fibrosis. The alveolar ducts are most commonly involved, although the main lactiferous ducts, the terminal ducts, and even acini may show dilatation. Numerous ducts or only one may be involved. The cells lining the ducts vary greatly in character. They may be cuboidal or columnar, and are often flattened. The cytoplasm is pale-staining and slightly basophilic with hematoxylin and eosin. The nuclei are deep-staining and round or oval. In many ducts the cells are greatly increased in number as well as in size, and coalesce to form projections, arches, or even solid sheets which may partially or completely fill the lumina. Papillary ingrowths into dilated ducts may be so atypical that only the absence of infiltrative growth indicates the benign nature of the process. These ingrowths were found in 68 per cent of our cases.

Acini as well as ducts are frequently involved. When distended, the epithelium of the acini is very low, appearing to be atrophic. Large retention cysts may be formed which may coalesce to form composite cysts.

There is an increase in the periductal and periacinar connective tissue, resulting in a diffuse fibrosis of the breast and causing wide separation of the lobules. The connective tissue may undergo hyaline degeneration when the fibrosis is of long standing and pressure atrophy of glandular tissue may result. The fibrosis may be associated with lymphocytic and/or plasma cell infiltration of the stroma, which may be localized in the region of the dilated ducts or may be diffusely scattered throughout the involved breast.

Hypertrophic pale-celled glandular epithelium, of the apocrine gland type, was found lining small glands and cysts of various sizes in approximately 60 per cent of our cases. In such areas the cuboidal or cylindrical cells are filled with closely packed eosinophilic granules or with a homogeneous eosinophilic cytoplasm. A small, round, poorly stained nucleus is located at the base of the cell. This epithelium is frequently thrown into folds and forms arches in the lumen. Around the base of the epithelium the layer of myoepithelium is thin as compared to that around the more normal secretory glands.

Glandular hyperplasia of the terminal ducts and acini was present in 62 per cent of our cases. There may be considerable increase in the number of acini in each lobule. Frequently the glandular hyperplasia is definitely atypical and the acini are filled with nests of cells. The periductal and periacinar fibrosis is particularly prominent in these areas. The abnormal response of the breast to excessive cyclical stimulation is evidenced by the dilatation of the terminal ducts and particularly of the acini. This is seen in scattered portions of the breast with normal breast tissue in the intervening portions, and also in breasts showing other cystic changes. The epithelial cells become swollen and tall, and there may be desquamated cells in the lumina of the ducts. There is also a definite periductal fibrosis with a localized lymphocytic infiltration.

Statistical Analysis

The material for this study was obtained from 330 women with lesions of the breast which necessitated operative removal of a specimen for microscopic examination during the period from July 1, 1937, to June 30, 1939. During this period 48 additional examples of carcinoma of the breast were seen; but these were excluded from further consideration because they were either prepared slides submitted from other institutions or small "biopsy" specimens not suitable for the purposes of this study. The material suitable for study may be tabulated as follows:

With carcinoma		118
(All mastectomies; 4 to 15 blocks of tissue examined from each breast.)		
Without carcinoma		212
Mastopathia cystica	82	
(Partial mastectomies, a few complete mastectomies and numerous generous "biopsy" specimens; 2 to 10 blocks examined from each patient.)		
Miscellaneous benign neoplasms, abscesses, and various other lesions	130	
Total		330

Definite evidence of coexisting mastopathia cystica was found in 67, or 56.7 per cent, of the 118 breasts removed for carcinoma, either in the immediate vicinity of the tumor or elsewhere in the breast. In 16 cases, 13.5 per cent, the carcinoma appeared to be arising in the area of mastopathia cystica. In these specimens the epithelial proliferation within the ducts was not only atypical but the basement membrane was frequently disrupted, and there was carcinomatous invasion of the surrounding tissues. Not only was there a close similarity between the infiltrating cells and the atypical papillary ingrowths within the dilated ducts, but also physical continuity between the two could be traced in frequent examples. In such there was ample evidence to justify the assumption that the carcinoma had arisen in the altered epithelium of mastopathia cystica. It will be recalled that in 68 per cent of the breasts with mastopathia cystica in this series, atypical papillary ingrowths into the dilated ducts were found. These were frequently associated with nests or solid sheets of cells completely filling the ducts. Only lack of evidence of infiltrative growth differentiated some of these areas from those known to be carcinomatous.

Since carcinoma of the breast and mastopathia cystica are both relatively common conditions, the possibility of their chance coincidence must be considered. Utilizing the basic information which follows, a fourfold table was arranged and the χ^2 test was applied to the 330 specimens examined (ignoring the 48 cases of carcinoma seen in "biopsy" material only).

Carcinoma, 118.
67 (57 per cent) also showed mastopathia cystica.
51 (43 per cent) showed no mastopathia cystica.
Mastopathia cystica, 149.
67 (45 per cent) also showed carcinoma.
82 (55 per cent) showed no carcinoma.
Neither carcinoma nor mastopathia cystica, 130.

The resulting fourfold table is as follows:

		Carcinoma: Present	Carcinoma: Absent	Total
Mastopathia cystica	Present	67	82	149
	Absent	51	130	181
	Total	118	212	330

For the above data,

$$\chi^2 = \frac{(330)[(67)(130)-(51)(82)]^2}{(118)(212)(149)(181)} = 10.029$$

This value for χ^2 indicates that the chances of getting the above results, if mastopathia cystica and carcinoma are independent, are of the order of 1 in 1,000 (27). Inasmuch as the normal range of probability by this test is 1 to 5 in 100, it is obvious that the association of the two conditions is highly significant.

SUMMARY

In a series of 330 consecutive surgical specimens of breast tissue, mastopathia cystica was found in 67 of a total of 118 with carcinoma. Application of the χ^2 test to the resulting data indicates that this degree of association cannot be expected through chance distribution and is statistically significant. On the basis of these findings, a causal relationship between mastopathia cystica and mammary carcinoma must be accepted. Although the highest incidence of mastopathia cystica is found in the decade of 40 to 50 years, the incidence of carcinoma of the breast continues at a high level for the next three decades. Any patient upon whom a diagnosis of mastopathia cystica is established should be followed carefully by repeated clinical examinations. If any change in the character of the lesion develops, histologic evidence of its nature should be considered essential.

I am indebted to Dr. Churchill Eisenhart of the University of Wisconsin, Madison, Wisconsin, for a critical evaluation of the statistical method.—Author.

REFERENCES

1. Berning, H., and Bücker, J. Mastopathia cystica (apokrine Drüsencysten) und Carcinom. Virchows Arch. f. path. Anat., **298**:728-742. 1936-37.
2. Bloodgood, J. C. Importance of Early Recognition and Operative Treatment of Malignant Tumors. Variation of the Extent of the Operative Removal According to the Relative Malignancy of the Tumor. J. A. M. A., **47**: 1470-1475. 1906.
3. Bloodgood, J. C. The Pathology of Chronic Cystic Mastitis of the Female Breast. With Special Consideration of Blue-Domed Cyst. Arch. Surg., **3**:445-542. 1921.
4. Bloodgood, J. C. Border-line Breast Tumors. Encapsulated and Non-encapsulated Cystic Adenomata, Observed from 1890 to 1931. Am. J. Cancer, **16**:103-176. 1932.
5. Campbell, O. J. The Relationship between Cystic Disease of the Breast and Carcinoma. Arch. Surg., **28**:1001-1056. 1934.
6. Charteris, A. A. On the Changes in the Mammary Gland Preceding Carcinoma. J. Path. & Bact., **33**:101-117. 1930.
7. Cheatle, G. L., and Cutler, M. Tumours of the Breast. Their Pathology, Symptoms, Diagnosis and Treatment. London: E. Arnold & Company. 1931.
8. Cutler, M. Relation of "Chronic Mastitis," Cysts and Papillomas to Cancer of the Breast. J. A. M. A., **99**: 2152-2157. 1932.
9. Dahl-Iverson, E., and Starup, U. Recherches sur la fréquence de la mastopathie kystique dans le carcinome mammaire. Lyon chir., **35**:570-573. 1938.
10. Deaver, J. B., and McFarland, J. The Breast: Its Anomalies, Its Diseases, and Their Treatment. Pp. 270-313. Philadelphia: P. Blakiston's Son & Company. 1917.
11. Dietrich, A., and Frangenheim, P. Die Erkrankungen der Brustdrüse. Neue Deutsche chir., **35**:1-309. 1926.

12. Ewing, J. Neoplastic Diseases. P. 467. Philadelphia: W. B. Saunders Company. 1919.
13. Fischer, W. Über die klinische und pathologisch-anatomische Beurteilung von Geschwülsten und zystischen Veränderungen der Brustdrüse. Deutsche Ztschr. f. Chir., **192**:1-12. 1925.
14. Greenough, R. B., and Simmons, C. C. Results of Conservative Treatment of Cystic Disease of the Breast. Ann. Surg., **60**:42-56. 1914.
15. Johnson, R. Some Clinical Aspects of Carcinoma of the Breast. Brit. J. Surg., **12**:630-649. 1924-25.
16. Keynes, G. Chronic Mastitis. Brit. J. Surg., **11**:89-121. 1923-24.
17. Kilgore, A. R. Breast Cysts and Cancer. Internat. S. Digest, **19**:195-201. 1935.
18. Klingenstein, P. Cystic Disease of the Breast. Ann. Surg., **101**:1144-1152. 1935.
19. Lewis, D., and Geschickter, C. F. The Relation of Chronic Cystic Mastitis to Carcinoma of the Breast. Surg., Gynec. & Obst., **66**:300-307. 1938.
20. MacCarty, W. C., and Mensing, E. H. IX. The Relation between Chronic Mastitis and Carcinoma of the Breast. Collected Papers of the Mayo Clinic, **7**:918-922. 1915.
21. Morpurgo, B. Beziehungen der Fibrosis cystica zu anderen Krankheiten der Brustdrüse. Verhandl. d. deutsch. path. Gesellsch., **25**:306-309. 1930.
22. Rodman, J. S. Chronic Cystic Mastitis. A Further Report on the Nature of the Process. Am. J. Surg., **28**:452-460. 1935.
23. Semb, C. Pathologico-anatomical and clinical investigations of fibro-adenomatosis cystica mammae and its relation to other pathological conditions in mamma, especially cancer. Acta chir. Scandinav. (supp. 10), **64**:1-484. 1928.
24. Warren, S. The Relation of "Chronic Mastitis" to Carcinoma of the Breast. Surg., Gynec. & Obst., **71**:257-273. 1940.
25. Whitehouse, H. B. Mastopathia and Chronic Mastitis. Surg., Gynec. & Obst., **58**:278-286. 1934.
26. Willer, H. Ueber die apokrinen Drüsen und den Krebs der weiblichen Milchdrüse. Zentralbl. f. allg. Path. u. path. Anat., **70**:49-55. 1938.
27. Yule, G. U., and Kendall, M. G. An Introduction to the Theory of Statistics. Eleventh edition. Table 4B, p. 535. London: C. Griffin & Company, Ltd. 1937.

Further Observation on Serum Acid Phosphatase Activity in Carcinoma of the Prostate

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During the past three years serum acid and alkaline phosphatase determinations have been carried out by Barringer and Woodard (1), Gutman and Gutman and their co-workers (2-4, 10), Huggins (7-9), etc., as a means of facilitating early recognition of metastatic bone lesions from carcinoma of the prostate. In recent publications (5, 6) we have pointed out that, in our opinion, serum acid phosphatase values up to 4.0 King-Armstrong units per 100 cc. of blood serum should be considered normal. Values of from 4.0 to 6.0 units represent borderline figures which have no appreciable diagnostic importance provided no further increase of the phosphatase level takes place on subsequent determinations. A considerable elevation of the serum acid phosphatase is always indicative of metastatic bone involvement from carcinoma of the prostate, and repeated determinations in these cases have shown that there is a definite tendency of the serum acid phosphatase activity to increase as the disease progresses.

Recent reports by Huggins and his co-workers (7-9) indicate that bilateral orchidectomy may result in appreciable improvement in cases of prostatic cancer even if demonstrable metastases to the bones are present. This improvement coincides with gain in weight, increase in the red blood count, regression in size and softening of the consistency of the prostate, and decrease of the serum acid phosphatase activity. Favorable results were also obtained by Huggins and his co-workers if the patients received stilbestrol medication.

It is obvious, therefore, that determinations of serum acid phosphatase activity are no longer of diagnostic value only. They are of value also as an objective indicator of the efficacy of castration or stilbestrol treatment of prostatic cancer, especially if metastatic bone involvement has occurred.

Up to the time of this writing, serum acid and alkaline phosphatase determinations have been carried out in 430 patients admitted to the New York State Institute for the Study of Malignant Diseases; 283 of these patients suffered from a variety of diseases other than prostatic cancer and in 147 patients carcinoma of the prostate was diagnosed.

The values for serum acid phosphatase activity in the group of 283 control cases were invariably below 6.0 King-Armstrong units. As a rule, determinations were carried out but once.

On the other hand, serum acid and alkaline phosphatase determinations were made routinely every 3 months or oftener in all cases of carcinoma of the prostate. In 42 cases our observations are based on data collected for more than one year.

Of the 147 patients with carcinoma of the prostate a positive biopsy was obtained in 105 instances. In the remaining 42 patients repeated attempts to obtain a satisfactory biopsy were unsuccessful in 21 instances and in 21 patients no biopsy was done for various reasons. In spite of that a diagnosis of carcinoma of the prostate was made from rectal and, frequently, cystoscopic examination. In all of these patients the prostate was definitely enlarged, indurated, and nodular, and infiltration into the pelvis with involvement of the seminal vesicles was present. Also partial encirclement of the rectum was felt in numerous patients of this group of 42.

No evidence of bone metastases was found in 100 of the 147 patients with carcinoma of the prostate. Seventy-seven had serum acid phosphatase levels below 4.0 King-Armstrong units once or on repeated determinations. In only 1 patient a serum acid phosphatase activity of more than 6.0 units was obtained (6.9 units). In the remaining 22 patients of this group serum acid phosphatase determinations yielded levels of from 4.0 to 6.0 units. In 8 of these 22, a tendency to gradual increase of the serum acid phosphatase activity was present, whereas in 14 instances a return to below 4.0 units was observed on subsequent determinations (see Table I).

TABLE I: SERUM ACID PHOSPHATASE DETERMINATIONS IN 147 PATIENTS WITH CARCINOMA OF THE PROSTATE

	Units of serum acid phosphatase *				
		4.0 to 6.0			
	Below 4.0	Tendency to increase	Returning to below 4.0	More than 6.0	Total
Patients with no evidence of metastases	77	8	14	1	100
Patients with bone metastases suspected clinically or roentgenologically	0	2	0	6	8
Patients with bone metastases demonstrable in x-ray picture	4	2	0	33	39
Total	81	10	14	42	147

*All phosphatase determinations were carried out by the King-Armstrong method.

Metastases were suspected from the clinical course in 8 patients, although metastatic bone lesions could not be demonstrated beyond doubt in this group. The suspicion of metastatic bone involvement was based on a rapid downhill course of the patients accompanied by increasing pain in the back and legs. Repeated blood chemistry determinations showed that none of these patients developed uremia. The serum acid phosphatase activity in these 8 subjects varied from 8.4 to 50.0 King-Armstrong units on last determination. Three of these 8 patients died 2 to 4 months after elevation of the serum acid phosphatase took place.

In this connection it is of interest that Simpson (11) was able to demonstrate, in a necropsy case of carcinoma of the prostate, extensive metastatic bone involvement microscopically, although metastatic bone changes could not be demonstrated roentgenologically. In our opinion it is probable that such microscopic bone involvement from prostatic cancer may occur more frequently than is indicated in the literature. Since determinations of serum acid phosphatase activity have been introduced as a routine procedure in cases of carcinoma of the prostate, metastases will be diagnosed on the living patient in numerous instances instead of at necropsy.

Definite metastases to the bones, demonstrable in the x-ray picture, were present in 39, or 26.5 per cent, of the 147 patients with prostatic cancer. As shown in Table I, 4 patients had serum acid phosphatase levels below 4.0 King-Armstrong units. Figures of from 4.0 to 6.0 units of serum acid phosphatase activity were determined in 2 others. The remaining 33 patients of this group had serum acid phosphatase values above 6.0 units. In 10 patients figures of more than 100 units were determined; the highest, obtained in 1 patient, amounting to 1,350 King-Armstrong units. Twenty-seven of these patients had demonstrable bone metastases and elevation of the serum acid phosphatase activity already present on admission. In the remaining 6, elevation of the serum acid phosphatase developed during the course of observation, and x-ray pictures, negative for metastatic lesions on admission, showed metastatic bone changes on subsequent examinations. In 3 of these 6 cases, elevation of the serum acid phosphatase activity preceded the roentgenological detection of bone metastases.

No relationship between the extent of metastatic involvement and the degree of serum acid phosphatase elevation could be established in our series of cases. In 4 patients, though almost the entire skeleton was involved by metastases, the serum acid phosphatase activity did not exceed 25 King-Armstrong units. On the other hand, metastatic lesions were confined to the pelvic girdle and the left femur in the patient who had a serum acid phosphatase level of 1,350 units.

If metastases to bones develop, with resulting elevation of the serum acid phosphatase, subsequent determinations show a definite tendency of the serum acid phosphatase activity to increase. This is either gradual or rapid, depending upon the activity of the metastatic lesion, until death occurs, provided no effective treatment is carried out. In 5 of the 33 patients with elevated serum acid phosphatase activity and demonstrable bone metastases we have had as yet no opportunity to repeat the determinations, either because the patients did not return or because they had been too recently admitted to our service. Follow-up determinations over a period of from 6 months to one year and 8 months in all of the remaining 28 cases yielded increasing values for serum acid phosphatase activity. The average serum acid phosphatase activity, which amounted to 36.4 King-Armstrong units on initial determination, yielded an average value of 120.7 units on last determination.

Although our patients with metastatic bone lesions causing elevation of the serum acid phosphatase activity number only 33, it is our impression that the degree of serum acid phosphatase elevation is a valuable help in prognosis. Of the 10 patients with serum acid phosphatase levels of more than 100 units, 2 were admitted only 2 and 4 weeks before the time of this writing. Of the remaining 8 patients, 5 died of the disease in an average time of 5.4 months. One case was lost trace of and only 2 patients, or 25 per cent, are alive. On the other hand, of 23 patients with serum acid phosphatase values ranging from 6.0 to 100 units, 12 died in an average time of 7.5 months and 11, or 47.8 per cent, are alive.

Our experiences with serum acid phosphatase determinations over a period of 2 years have confirmed our previous statements, which were that marked elevation of the serum acid phosphatase level is indicative of metastatic bone involvement from carcinoma of the prostate. Definitely elevated values for serum acid phosphatase were obtained in 33, or 84.7 per cent, of 39 patients with demonstrable metastatic bone changes. This ratio is analogous to the data presented by the Gutmans and their co-workers (4), who found increased serum acid phosphatase levels in 37, or 84.1 per cent, of 44 cases with metastatic bone lesions from prostatic cancer.

As has been stated in our previous publications (5, 6) we are of the opinion that figures for serum acid phosphatase activity up to 4.0 King-Armstrong units should be considered normal.

The interpretation of borderline figures between 4.0 and 6.0 King-Armstrong units is difficult in many instances. Of 22 patients without evidence of bone metastases, in whom such borderline values were obtained once or repeatedly, figures below 4.0 units were obtained on subsequent determinations in 14 cases. In

only 8 patients of this group was the level of from 4.0 to 6.0 units maintained, and it remains to be seen whether a further increase coinciding with roentgenological evidence of metastatic bone involvement will take place in these 8. The utmost conservatism should be used in the interpretation of these borderline figures, and final judgment withheld until follow-up determinations and the study of repeated x-ray pictures reveal whether the slight elevation was indicative of early metastatic bone involvement or of hemolysis or other extraneous factors.

It has been suggested by Huggins and his collaborators (9) that 10.0 King-Armstrong units should be regarded as the upper limit for serum acid phosphatase activity. However, our studies have convinced us that figures above 6.0 units represent an elevation of the serum acid phosphatase level which has diagnostic value. Serum acid phosphatase values ranging from 6.0 to 10.0 units were found once or repeatedly in 18 of our 147 cases with prostatic cancer. Only 1 patient with a serum acid phosphatase activity of 6.9 King-Armstrong units showed no evidence of bone metastases, and 4 patients, in whom metastases were suspected clinically or roentgenologically, showed a further increase of the serum acid phosphatase activity to values above 10.0 units. In the remaining 13 cases of this group evidence of metastatic bone involvement was demonstrable in the x-ray picture before the serum acid phosphatase activity exceeded 10.0 units on later determinations.

In concluding we wish to emphasize that the data presented are limited to figures obtained on patients prior to castration or stilbestrol medication. Studies in this field have been in progress on our service during the past 5 months, and the results will be reported as soon as sufficient time has elapsed to permit conclusions, based on a longer period of observation.

SUMMARY

Data of serum acid phosphatase determinations collected over a period of 2 years are presented. Two hundred and eighty-three of 430 patients in whom serum acid phosphatase determinations were carried out were control cases. In the remaining 147 patients carcinoma of the prostate was diagnosed.

Serum acid phosphatase levels up to 4.0 King-Armstrong units should be considered normal. Values of from 4.0 to 6.0 units represent borderline figures which are only diagnostic for metastatic bone lesions from carcinoma of the prostate if further elevation takes place. A considerable rise in the serum acid phosphatase activity is indicative of bone metastases from prostatic cancer. With one exception, such an elevation was not encountered in the group of controls or in patients not suspected of having bone metastases from carcinoma of the prostate.

Serum acid phosphatase determinations are a valuable diagnostic aid in the early recognition of bone metastases from prostatic cancer in new patients and in patients who are under constant observation.

If elevation of the serum acid phosphatase activity develops, there is a tendency to steady increase provided no effective treatment is initiated.

A relationship between the degree of serum acid phosphatase elevation and the extent of metastatic bone involvement could not be established in our series of cases.

Serum acid phosphatase levels of more than 100 King-Armstrong units have to be regarded as an unfavorable prognostic sign.

It is emphasized that the determination of serum acid phosphatase activity is also of value as an objective indicator of the effectiveness of castration or stilbestrol in the treatment of prostatic cancer, as suggested by Huggins and his co-workers (7-9). This is especially true for patients with metastatic bone lesions causing elevation of the serum acid phosphatase activity.

REFERENCES

1. Barringer, B. S., and Woodard, H. Q. Prostatic Carcinoma with Extensive Intra-Prostatic Calcifications. Tr. Am. A. Genito-Urin. Surgeons, **31**:363-369. 1938.
2. Gutman, A. B., and Gutman, E. B. "Acid" Phosphatase and Functional Activity of the Prostate (Man) and Preputial Glands (Rat). Proc. Soc. Exper. Biol. & Med., **39**:529-532. 1938.
3. Gutman, E. B., Sproul, E. E., and Gutman, A. B. Significance of Increased Phosphatase Activity of Bone at the Site of Osteoplastic Metastases Secondary to Carcinoma of the Prostate Gland. Am. J. Cancer, **28**:485-495. 1936.
4. Gutman, A. B., Gutman, E. B., and Robinson, J. N. Determination of Serum "Acid" Phosphatase Activity in Differentiating Skeletal Metastases Secondary to Prostatic Carcinoma from Paget's Disease of Bone. Am. J. Cancer, **38**:103-108. 1940.
5. Herger, C. C., and Sauer, H. R. Serum Acid Phosphatase Determination in Carcinoma of the Prostate. Urol. & Cutan. Rev., **45**:283-285. 1941.
6. Herger, C. C., and Sauer, H. R. Relationship of Serum Acid Phosphatase Determination to Presence of Bone Metastases from Carcinoma of Prostate. J. Urol., **46**:268-302. 1941.
7. Huggins, C., and Hodges, C. V. Studies on Prostatic Cancer. I. The Effect of Castration, of Estrogen and of Androgen Injection on Serum Phosphatases in Metastatic Carcinoma of the Prostate. Cancer Research, **1**:293-297. 1941.
8. Huggins, C., Stevens, R. E., and Hodges, C. V. Studies on Prostatic Cancer. Arch. Surg., **43**:209-223. 1941.
9. Huggins, C., Scott, W. W., and Hodges, C. V. Studies on Prostatic Cancer. III. J. Urol., **46**:997-1006. 1941.
10. Robinson, J. N., Gutman, E. B., and Gutman, A. B. Clinical Significance of Increased Serum "Acid" Phosphatase in Patients with Bone Metastases Secondary to Prostatic Carcinoma. J. Urol., **42**:602-618. 1939.
11. Simpson, W. M. Diffuse Vertebral Metastasis of Prostatic Carcinoma without Bony Changes. Am. J. Roentgenol., **15**:534-541. 1926.

Blood Cell Factors in the Metastasis of the Brown-Pearce Tumor*

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It has been a moot point whether the cellular reaction in the blood or in the tissues about spontaneous or transplanted neoplasms bore any causal relation to their regression, or whether the lack of a proper reaction was instrumental in their continued growth and spread (10).

A new approach to the problem was designed to add information on this point. Its essential feature was determination of the average levels of nine blood cell factors in apparently healthy male rabbits of comparable ages, and the discard of all animals in which any single level was abnormal; *i.e.,* more than 1.96 standard deviations above or below the standard means for the rabbit species. In this way 45 per cent of an apparently healthy population was rejected for the purpose of the study and the remaining animals were then inoculated intratesticularly with the Brown-Pearce tumor. Obviously the pretransplantation levels, perhaps largely hereditary (2, 3), could not be the result of the subsequent growth and spread of the tumor. A preceding paper (4) dealt with the relation of the nine pretransplantation blood factor levels to the success of transplantation of this neoplasm, and three of the nine factors were significantly related to the success of transplantation; *viz.,* the red blood cell, the hemoglobin, and the total white blood cell levels. The present communication is concerned with the relation of the pretransplantation levels of the same nine factors to the spread of, and the mortality from, the neoplasm in the animals successfully inoculated.

MATERIALS AND METHODS

In a preceding paper (4) the success of intratesticular transplantation of the Brown-Pearce tumor was studied in 108 rabbits selected with respect to the pretransplantation levels of nine blood factors. The blood factors were: the average levels of the red blood cells, the blood platelets, the hemoglobin, the total white blood cells, the neutrophiles, the basophiles, the eosinophiles, the lymphocytes, and the monocytes. Transplantation was considered to have been successful, first, if a progressive growth appeared at the site of inoculation of the tumor emulsion into the (right) testis and persisted for more than 30 days; or, second, if by the end of 60 days (at which time all the animals which had not died were sacrificed) tumor tissue was found anywhere in the body, regardless of whether or not a nodule had appeared at the site of inoculation.

The 108 animals used were drawn from 195 young adult male rabbits chosen on the basis of apparent health and maturity. Seven animals were discarded because of the development of intercurrent disease, one because necropsy had not been performed, and 79 others because one or more of the nine blood factor levels was more than 1.96 times the standard deviation above or below the standard mean for the rabbit species. Eighteen animals were further discarded for the purpose of this paper because transplantation had not been successful in them.

Although blood examinations were made before inoculation on each of the 90 animals considered to have been successfully inoculated, blood hemoglobin estimations were available on only 84 and blood platelet counts on only 63. The absolute numbers of the various white cells per cubic millimeter were used.

The 90 animals successfully inoculated were from 17 experiments each of which was terminated 2 months after intratesticular transplantation of the Brown-Pearce tumor. The animals, according to the course of the disease, fell into three categories: First, in 16 there was growth of vigorous primary tumors which persisted for more than 30 days but failed to develop metastases during the 2-month period. At necropsy the primary tumors of each of these had regressed and in the case of 8 had completely disappeared except for small scars. Second, 35 developed metastases from which they died during the observation period. Third, the remaining 39 animals developed metastases but survived the observation period.

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The pretransplantation blood levels were divided into two categories for each factor in two different ways: First, the standard mean was taken as a point of division and the animals grouped according to whether the particular factor level was above or below the mean, the level being called high or low respectively. Second, the intermediate distance (that is, the standard deviation times 0.6745) above or below the mean was taken as a point of division, and the animals were grouped according to whether the particular level was within the intermediate distance or beyond this distance. The intermediate distance, like the mean, divided the observations into two approximately equal groups. The distributions of most of the blood factors studied were somewhat skewed and the means and modes did not correspond, but no significant relationships were lost as the data in all cases were minutely checked by the construction of correlation tables of various sorts.

The standard means and intermediate distances employed for the various blood cell factors were as follows: 5,370,000, 4,999,000, and 5,741,000 red cells; 69.1, 64.7, and 73.48 per cent hemoglobin; 566,000, 486,000, and 645,600 blood platelets; 7,690, 6,683, and 8,697 white blood cells; 3,780, 3,038, and 4,522 neutrophiles per cu. mm.; 500, 331, and 669 basophiles; 111, 50, and 172 eosinophiles; 2,550, 1,855, and 3,245 lymphocytes; 740, 517, and 963 monocytes (3).

The 90 animals were distributed according to the three tumor and the various blood factor categories. The expected values on the basis of a purely accidental distribution were calculated and χ^2 (5, 6, 8) determined for each factor grouping. χ^2 is a means of testing (when the number of expected observations is at least five in each subcategory or box) the statistical significance of observed departures from the expected.

In addition, the means of the pretransplantation blood factor levels were computed and compared for the animals in each of the three tumor categories. The standard error of the means was computed from the standard deviations obtained in an earlier study of normal male rabbits.

The mortality in percentage for various days after inoculation was plotted for each factor: first, according to groups of rabbits with high and low pretransplantation blood factor levels; and, second, according to intermediate and extreme levels.

Inasmuch as the 2-month observation period was an arbitrary interval which influenced the mortality values, another estimate of the spread of the neoplasm was indicated. The number of metastatic foci per animal successfully inoculated was employed. This is another criterion of the spread of the Brown-Pearce tumor which is equally dependable with the mortality curve. A metastatic focus is defined as the presence of one or more metastases within one of the 50 areas into which the animal body was divided for recording post-mortem examinations. The number of metastatic foci was computed for each of the blood factor categories (high, low, intermediate, and extreme) and for the animals surviving as well as for those dying with metastases.

RESULTS

Among the 90 animals successfully inoculated with the Brown-Pearce tumor no significant correlation existed between the pretransplantation blood levels of the hemoglobin, the total white cells, the neutrophiles, the lymphocytes, or the monocytes and, first, the incidence of metastases, second, the mortality from the neoplasm, or third, the number of metastatic foci developed (Tables I, II, III). The preinoculation red cell, platelet, basophile, and eosinophile levels, however, were significantly related to the spread of the Brown-Pearce tumor in animals successfully transplanted (Tables I, II, III).

Intermediate values of the red blood cell and basophile pretransplantation levels were associated with fewer metastases and a lower mortality from the neoplasm than were extreme values. The association of extreme eosinophiles with resistance was not so great as the association of high (above mean) eosinophiles with the same factor, and was found to be due to the skew distribution of the eosinophiles upwards and the relative scarcity of low extreme eosinophile values.

High eosinophile and low blood platelet pretransplantation levels were associated each with fewer metastases and a lower mortality in animals successfully transplanted. Further data on the association of the eosinophile, basophile, and platelet levels to the reaction of the rabbit to this neoplasm will be presented elsewhere.

DISCUSSION

The results in the present study confirm and amplify preliminary observations that the blood eosinophile level is related to the host reaction to the Brown-Pearce tumor (1). In the preliminary study the success of transplantation was not analyzed separately from the growth and spread of the tumor among animals successfully inoculated, and standard values for the various blood factors were not available for the rabbit. Furthermore, rigid selection on the basis of blood levels was not employed, as in the present study; for this reason only 35 of the animals used in the original study are included in the present study, the others having been discarded because of this objective manner of selection.

TABLE I: NINETY RABBITS SUCCESSFULLY INOCULATED WITH THE BROWN-PEARCE TUMOR DISTRIBUTED ACCORDING TO PRETRANSPLANTATION BLOOD CELL FACTOR LEVELS AND SUBSEQUENT COURSE OF THE NEOPLASM

Blood factor level		R #	H #	BP #	W #	N #	B #	E #	L #	M #
High	DM	14	11	12	15	16	21	13	16	14
	SM	19	13	8	19	17	21	25	19	16
	SP	6	5	4	8	7	8	14	5	8
Low	DM	21	21	9	20	19	14	22	19	21
	SM	20	25	26	20	22	18	14	20	23
	SP	10	9	4	8	9	8	2	11	8
χ^2		..	..	7.94	..	..	..	12.56	..	..
P		..	..	0.02	..	..	..	0.001	..	..
Intermediate	DM	16	15	10	21	23	17	25	21	20
	SM	24	18	18	19	24	14	30	22	23
	SP	11	9	2	9	10	12	7	10	9
Extreme	DM	19	17	11	14	12	18	10	14	15
	SM	15	20	16	20	15	25	9	17	16
	SP	5	5	6	7	6	4	9	6	7
χ^2		..	..	..	..	..	6.96	5.99	..	..
P		..	..	..	..	..	0.03	0.05	..	..

Note: #, number of rabbits; R, red blood cells; H, hemoglobin (available on 84 animals only); BP, blood platelets (available on 63 animals only); W, white blood cells; N, neutrophiles; B, basophiles; E, eosinophiles; L, lymphocytes; M, monocytes; high mean, +0.0–1.96 standard deviations; low mean, −0.0–1.96 standard deviations; intermediate mean, ±0.0–0.67 standard deviations; extreme mean, ±0.67–1.96 standard deviations; DM, dying with metastases; SM, surviving with metastases; SP, surviving with primary tumor only; χ^2, see Fisher (6); P, probability (0.01, statistically significant; 0.01–0.05, probably significant).

TABLE II: PRETRANSPLANTATION BLOOD CELL FACTOR LEVELS AMONG 90 RABBITS SUCCESSFULLY INOCULATED WITH THE BROWN-PEARCE TUMOR, AVERAGED ACCORDING TO THE COURSE OF THE DISEASE

		R	H	BP	W	N	B	E	L	M
Primary tumor only	n	16	14	8	16	16	16	16	16	16
	m	529	66.6	532	716	371	52	18	237	72
	s_m	14	1.8	45	47	28	6	2	27	9
Surviving with metastases	n	39	38	34	39	39	39	39	39	39
	m	540	67.0	511	765	359	55	13	260	74
	s_m	9	1.1	20	29	18	4	1	17	5
Dying with metastases	n	35	32	21	35	35	35	35	35	35
	m	532	66.6	582	747	370	57	11	241	68
	s_m	9	1.2	26	31	19	4	1	18	6
Differences in mean blood factor levels		...	...	Sig	...	...	...	Sig	...	...

Note: R, red cells (0000 omitted); H, hemoglobin (Newcomer in per cent); BP, platelets (000 omitted); W, total white cells (0 omitted); N, B, E, L, M, neutrophiles, basophiles, eosinophiles, lymphocytes, monocytes in absolute numbers per cu. mm. of blood (0 omitted); n, number of rabbits, m, mean of blood factor levels, s_m, standard error of the mean; Sig, statistically significant difference between the tumor categories.

TABLE III: NUMBER OF METASTATIC FOCI AMONG 90 ANIMALS SUCCESSFULLY INOCULATED WITH THE BROWN-PEARCE TUMOR, DISTRIBUTED ACCORDING TO PRETRANSPLANTATION BLOOD FACTOR LEVELS

Means		R #	H #	BP #	W #	N #	B #	E #	L #	M #
MF	I	8.85	10.12	9.39	10.58	10.08	9.48	10.36	11.05	10.32
	Ex	12.38	11.00	11.85	10.35	11.19	11.19	10.79	9.68	10.70
	F	4.07	....	....	....	....	....	....	....	....
	P	0.05								
IS	I	6.94	8.39	8.76	8.63	8.49	6.84	9.19	8.96	8.73
	Ex	10.79	9.43	9.70	8.58	8.82	10.23	7.32	8.11	8.45
	F	6.14	....	....	....	....	4.16	....	....	....
	P	0.02	....	....	....	....	0.06	....	....	....
MF	L	10.93	10.26	8.86	11.18	10.05	10.38	11.72	11.18	10.30
	H	9.91	11.17	13.65	9.65	11.03	10.55	9.29	9.69	10.73
	F	....	....	4.84	....	....	....	....	....	....
	P	....	....	0.04	....	....	....	....	....	....
IS	L	8.78	8.58	7.95	9.31	8.44	8.30	11.11	8.72	8.71
	H	8.38	9.24	11.38	7.81	8.83	8.86	6.79	8.48	8.47
	F	....	....	....	....	....	....	6.74	....	....
	P	....	....	....	....	....	....	0.01	....	....

Note: #, number of metastatic foci; R, red cells; H, hemoglobin; BP, platelets; W, white cells; N, neutrophiles; B, basophiles; E, eosinophiles; L, lymphocytes; M, monocytes; I, intermediate; Ex, extreme; L, low; H, high; MF, animals with metastases; IS, animals successfully inoculated; F, variance between compared with variance within the means, see Snedecor (9); P, probability.

The hemoglobin and total white blood cell levels, which were related to the success of transplantation, were apparently unrelated to the course of the tumor in animals successfully inoculated. The eosinophile, the blood platelet, and the basophile levels (related to the growth and spread of the neoplasm) seemed to bear no relation to the success of transplantation. The red blood cell level was related both to the success of transplantation (secondary to the hemoglobin values) and to the spread of the tumor in animals successfully inoculated. These blood studies emphasize the belief that the host factors involved in the successful transplantation of a neoplasm are not necessarily those involved in its continued growth and spread.

The uniformity of the results obtained reflects the importance of a rigid selection of animal material and the employment, so far as is possible, of such objective methods of observation and analysis as were used. Another point of importance is the repetition of experiments, which numbered 17 in the present instance.

The neutrophile, basophile, eosinophile, lymphocyte, and monocyte levels were calculated in relative percentages as well as in absolute numbers per cubic millimeter for each of the 90 animals used. No relationship of the pretransplantation levels in relative percentage (except that of the eosinophile) to the success of transplantation, or to the growth and spread of the neoplasm in animals successfully inoculated, was noted. Correlation tables, high and low values, intermediate and extreme values, were employed. This is not compatible with Irwin's suggestion that blood differential counts be expressed in relative percentages in such studies (7).

The present study employed standard values for constitutional factors to eliminate those technical, pathologic, and physiologic variations in the animal population which were unrelated to the reaction of the rabbit to the neoplasm.

SUMMARY AND CONCLUSIONS

One hundred and eight rabbits apparently free from intercurrent disease and having each of nine blood factor levels within normal limits were inoculated intratesticularly with the Brown-Pearce tumor. In 90 the grafts were successful.

Among the 90 animals successfully inoculated, high eosinophile and low blood platelet pretransplantation levels were associated each with fewer metastases and a lower mortality than was the case with low eosinophile and high blood platelet values. Intermediate values of the red blood cell and basophile pretransplantation levels were associated with fewer metastases and a lower mortality from the neoplasm than were extreme values.

No significant correlation existed between the pretransplantation blood levels of the hemoglobin, the total white blood cells, the neutrophiles, the lymphocytes, or the monocytes and: (a) the incidence of metastases, (b) the mortality from the neoplasm, or (c) the number of metastatic foci developed.

The host factors which influenced the success of transplantation of the Brown-Pearce tumor were different from those which influenced its continued growth and spread and the mortality from the neoplasm.

REFERENCES

1. Casey, A. E., and Pearce, L. Studies on the Blood Cytology of the Rabbit. VIII. The Blood of Normal Rabbits as an Index of Their Resistance to a Transplantable Neoplasm. J. Exper. Med., **54**:475-492. 1931.
2. Casey, A. E., Rosahn, P. D., Hu, C. K., and Pearce, L. Hereditary Variations in the Blood Cytology of Normal Rabbits. Science, **79**:189-190. 1934.
3. Casey, A. E., Rosahn, P. D., Hu, C. K., and Pearce, L. Hemocytological Constitution of Adult Male Rabbits from Fifteen Standard Breeds. J. Exper. Med., **64**:453-469. 1936.
4. Casey, A. E., Pearce, L., and Rosahn, P. D. The Association of Blood Cell Factors with the Transplantability of the Brown-Pearce Tumor. Cancer Research, **2**:284-289. 1942.
5. Fisher, R. A. On the Interpretation of χ^2 from Contingency Tables, and the Calculation of P. J. Roy. Statist. Soc., **85**:87-94. 1922.
6. Fisher, R. A. Statistical Methods for Research Workers, Fourth edition. Edinburgh: Oliver and Boyd. 1932.
7. Irwin, M. R., and Bell, F. N. Studies on the Variation of the Blood Cells of Cattle in Health and during Brucella Infections. J. Infect. Dis., **63**:263-268. 1938.
8. Pearson, K. On the Criterion That a Given Series of Deviations from the Probable in the Case of a Correlated System of Variables Is Such That It Can Be Reasonably Supposed to Have Arisen from Random Sampling. Phil. Mag., Series V, **50**:157-175. 1900.
9. Snedecor, G. W. Calculation and Interpretation of Analysis of Variance and Covariance. Ames, Iowa: Collegiate Press, Inc. 1934.
10. Woglom, W. H. Immunity to Transplantable Tumours. Cancer Rev., **4**:129-214. 1929.

Transamination in Liver from Rats Fed Butter Yellow*

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The demonstration by Kinosita (11) that feeding butter yellow (*p*-dimethylaminoazobenzene) to rats on a deficient diet caused the production of liver cancer, provided a valuable experimental setup for comparing normal and malignant metabolism. The resulting butter yellow liver tumors are usually either cholangiomas or hepatomas, and thus their metabolism can be compared more appropriately with normal liver. In addition, it is possible to follow the metabolism of the liver during the period of transformation to malignancy. However, whether the intermediate stages are represented by increasing numbers of malignant cells admixed with more or less normal liver cells, or by different stages of a precancerous condition of all the cells, is not known.

In a previous publication (4) it was reported that different mouse tumors, fetal cat tissues, and regenerating rat liver showed a decreased transaminase activity. While the transaminase activity of the fetal liver, kidney, and brain was compared with that of the corresponding adult tissues, and the regenerating liver with normal liver, it was not possible to compare satisfactorily the mouse tumors with related normal tissues. It thus seemed desirable to study transaminase activity in a malignant tissue which arose from a normal tissue of known transaminase activity. In this paper experiments are reported in which the transaminase activity was measured in livers from rats fed butter yellow for varying lengths of time up to 200 days.

Feeding Experiments with Butter Yellow

Fifty albino Sprague-Dawley rats, 110 to 150 gm. in weight, were divided into 4 groups and placed on the following diets: [1]

1. 29 animals (15 males, 14 females)
   Brown unpolished rice ..... 800 gm.
   Butter yellow (3% in olive oil) ..... 20 gm.
   Vitamin-free casein (Labco) ..... 180 gm.
   Fresh carrot (one slice daily)
2. 10 animals (8 males, 2 females)
   Same as diet 1, but without casein
3. 6 animals (4 males, 2 females)
   Same as diet 1, but without butter yellow
4. 5 animals (3 males, 2 females)
   Same as diet 1, but without casein or butter yellow.

The animals consumed 6 to 7 gm. of the diets daily. This represents a daily butter yellow intake, in the case of the animals on diets 1 and 2, of 3.5 to 4.0 mgm. All the animals lost weight during the first 3 months, those on diets 1 and 3 losing less weight than the animals on diets 2 and 4. Aside from this, there was but slight difference in the ultimate course in the animals on diets 1 and 2, or 3 and 4.

Of the 39 animals receiving butter yellow, 13 died during the experiment. Five died between 20 and 30 days after it had been begun. Their livers showed no remarkable gross or histological changes. Two died after 34 and 41 days. Their livers exhibited, chiefly, early signs of cirrhosis with some periportal lymphocytic infiltration. One died after 87 days. Its liver had, in the gross, a hobnailed appearance, and histologically showed nodular cirrhosis with hyperplasia. The other 5 animals died between 122 and 185 days. All their livers showed gross and histological evidence of advanced nodular cirrhosis with varying amounts of bile duct adenomatosis. Three had many small areas of cholangioma and hepatoma. The death rates of the animals receiving butter yellow were: 13 per cent during the first 30 days, 18 per cent during the first 60 days, and 33 per cent during the 200 days of the experiment. Sugiura and Rhoads (16) reported 3 per cent mortality during the first month and 15 per cent during the first 60 days.

The gross and histological changes in the livers of 20 animals on diets 1 and 2 for 60 to 200 days were observed.[2] The changes were more or less typical of those reported by Orr (14) and by Sugiura and Rhoads (16). Between 60 and 100 days the chief histological changes were those of cirrhosis with varying amounts of bile duct adenomatosis. Between 100 and 200 days increasing amounts of malignant tissue were seen. The malignant changes were chiefly of the mixed type, with areas of both cholangioma and hepatoma. Small areas of cystadenoma were also occasionally seen during this interval.

The findings for these 20 animals are summarized in Table I. As can be seen, practically all the animals receiving butter yellow for 100 to 200 days showed evidence of hepatic malignancy.

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[1] We are indebted to Dr. K. Sugiura for advice on the preparation of these diets.

[2] We are indebted to Miss H. Ritter, Laboratory of Physiological Chemistry, Yale University, for the preparation of the histological sections.

Attempts to transplant 4 different hepatic tumors were unsuccessful. Histologically these tumors appeared highly malignant. One successful take failed to grow on a subsequent transplant.

Of the 11 animals on control diets 3 and 4, 1 died of undetermined cause.

TABLE I: THE EFFECT OF DIETS 1 AND 2 ON BODY WEIGHT AND LIVER HISTOLOGY

Animal	Diet	Initial weight, gm.	Final weight, gm.	Days on butter yellow	Liver findings *
1	1	122	120	60	—
2	1	130	100	75	—
3	1	139	76	85	—
4	1	144	104	90	—
5	2	138	94	93	—
6	1	150	120	96	(+?)
7	1	120	84	102	(+?)
8	1	122	110	108	+
9	1	124	78	112	—
10	2	138	84	126	+
11	2	112	62	132	++
12	1	112	70	140	+
13	1	129	94	150	++
14	2	118	80	167	+
15	2	136	90	175	++
16	1	118	95	182	++
17	1	140	104	195	++
18	1	138	100	199	++
19	1	135	123	200	+++
20	1	112	96	200	++

* — indicates no histological evidence of malignancy; +, ++, +++ indicate definite histological evidence of malignancy in increasing amounts.

DETERMINATION OF TRANSAMINASE ACTIVITY

Substrates.—The substrates used were *l*(+)-glutamic acid plus oxalacetic acid in equimolar concentrations (final concentration, 0.017 M, unless otherwise indicated). It has been shown (3–5) that this represents the most active transaminating system. The substrates were neutralized to pH 7.4 before addition.

Analytical methods.—Transamination was measured by determining the amount of α-ketoglutaric acid formed according to equation 1, using the method of Krebs (12). In some instances aspartic acid formation was also measured (2). The two methods checked well. The suitability of these analytical methods and the details of their application have been discussed (2).

Transaminase activity is expressed as per cent conversion of glutamic acid to α-ketoglutaric acid or aspartic acid (see equation 1).

$$l(+)\text{-glutamic acid} + \text{oxalacetic acid} \rightleftharpoons \begin{matrix}\alpha\text{-ketoglutaric acid} \\ + \\ \text{aspartic acid}\end{matrix} \quad [1]$$

EXPERIMENTAL PROCEDURE

The animals were killed by a blow on the head and exsanguinated. The liver was rapidly removed and placed in ice cold saline. Representative portions of the liver were dried on filter paper, weighed, and then homogenized with 9 parts of 0.1 M phosphate buffer, pH 7.4. The homogenization was carried out at ice bath temperature. The homogenate was diluted with phosphate buffer to give the required final concentrations.

Each incubation flask contained 3 ml. of homogenate plus 0.3 ml. of 0.2 M *l*(+)-glutamic acid. 0.3 ml. of 0.2 M oxalacetic acid, and 0.3 ml. of 0.1 M phosphate buffer were added to the experimental and control flasks respectively. Incubation was carried out with shaking at 38° C. for 15 minutes. With tissue concentrations greater than 1:80, anaerobic conditions were maintained by means of nitrogen gas and yellow phosphorus. Otherwise, incubations were carried out in air.

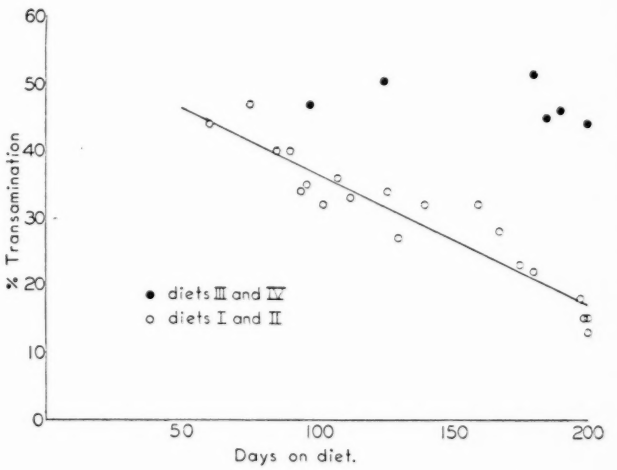

FIG. 1.—Relationship of days of butter yellow feeding to percentage transamination. See text for explanation.

RESULTS

RELATIONSHIP OF DAYS OF BUTTER YELLOW FEEDING TO PERCENTAGE TRANSAMINATION

In Fig. 1 the transaminase activity of livers from animals receiving butter yellow is shown. The solid circles represent determinations on livers from animals receiving diets 3 and 4; the open circles, determinations on animals receiving diets 1 and 2. All these points represent determinations on different livers at a tissue concentration of 1:80. As has been shown (5), a better comparative estimate of transaminase activity is obtained with this concentration of tissue, since at higher concentrations the rate of transamination is not proportional to the tissue concentration, due to the high transaminase content.

The regression line in Fig. 1 was calculated from the experimental points according to the method of Fisher (6) and is represented by the equation, $Y = 56.6 - 0.20$ days. That is, with each day of butter yellow feeding there is a decrease of 0.20 per cent transamination. The relation of percentage transamination to days of butter yellow feeding has a high correlation coefficient, r being equal to 0.94 (upper $r = 0.99$, lower $r = 0.77$).

From Fig. 1 it is apparent that the transaminase activity decreases progressively with continued ingestion of butter yellow to a low point at 200 days of feeding. The transaminase activity after 200 days is of the same order as that found in mouse tumors and embryonic tissue (4). It is of interest to note that although the livers show signs of malignant changes after about 125 days of butter yellow feeding, the transaminase activity continues to decrease with continued feeding. Whether this is due to the continued feeding of butter yellow or to the progressive development of the malignant changes is not apparent from these data. In all probability this is due to the fact that an increasing number of liver cells are undergoing malignant change. The livers from animals killed after 200 days all showed massive tumors with only small amounts of normal-appearing liver. This fact is brought out more clearly in Fig. 2, which shows the dilution curves for some of these livers.

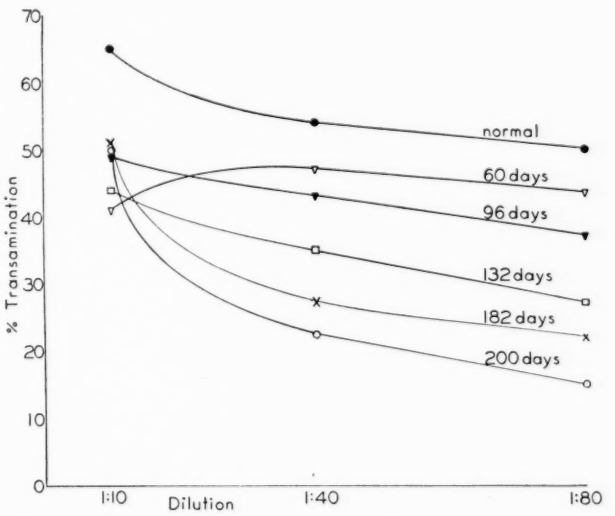

FIG. 2.—Transaminase activity at different dilutions during course of butter yellow feeding. Days of butter yellow feeding indicated on each curve.

It is obvious from Fig. 1 that there is very little change in transaminase activity in livers from animals on the control diets 3 and 4.

Effect of Dilutions on Transaminase Activity

In Fig. 2 dilution curves are shown which represent determinations on livers taken from animals after the indicated numbers of days of butter yellow feeding. The livers selected represented typical intermediate histological stages from early cirrhosis in the case of the 60-day liver to extensive cholangiomatous and hepatomatous changes in the case of the 200-day liver.

In the case of the 60-day liver the percentage transamination appears to increase upon dilution from a tissue concentration of 1:10 to 1:40. This at first suggested that butter yellow, or a metabolic intermediate, was inhibiting transaminase, and that this inhibiting effect was removed upon dilution. To test this, homogenized normal rat liver, in concentrations of 1:10, 1:40, and 1:80, was incubated anaerobically with and without butter yellow (10 mgm.) in the presence of $l(+)$-glutamic acid and oxalacetic acid. The percentage transamination was found to be no lower in the presence of butter yellow than in its absence. To test the possibility that this inhibition was due to an intermediate oxidation product, normal rat liver slices (3 gm.) were shaken in air for 30 minutes in the presence and absence of 50 mgm. of butter yellow. The slices and medium were then homogenized and aliquots incubated anaerobically with $l(+)$-glutamic acid plus oxalacetic acid. There was no evidence of inhibition due to the presence of butter yellow.

From these experiments it is apparent that neither butter yellow itself nor any oxidation product formed under these conditions is responsible for this inhibitory effect.

It is further seen from Fig. 2 that the percentage transamination at a concentration of 1:10 does not decrease notably with further butter yellow feeding. This would imply that the concentration of inhibitor(s) does not increase beyond a certain level which is insufficient to inhibit completely the relatively large amount of transaminase present. Apparently this level of inhibitor is reached after 60 days of butter yellow feeding. It is obvious that the amount of transaminase present at 1:10 dilution is still large enough to catalyze the reaction at a considerable rate. That there is less transaminase actually present, however, is apparent from the dilution curves (Fig. 2) which show that after 200 days of butter yellow feeding there is approximately only one-third the amount of transaminase present as compared with normal liver.

The progressive decrease in transaminase activity with butter yellow feeding would be expected to result eventually in dilution curves similar to those previously reported for transplanted mouse tumors (4) if a low transaminase content is a characteristic of tumors. Actually the 182- and 200-day curves closely resemble those reported for mouse tumors, except that in the case of the butter yellow tumors the 1:10

dilution values are higher. Inasmuch as there still was some normal liver tissue admixed with the butter yellow tumors this is to be expected. It is highly probable that after transplantation these liver tumors would show the same low transaminase content at 1:10 dilution.

The Effect of Certain Possible Metabolic Intermediates of Butter Yellow on Transaminase Activity

The inhibitory effect of certain of the possible metabolic intermediates of butter yellow on rat heart muscle transaminase is shown in Table II. Quinone appears to be the most potent inhibitor, while

Table II: The Effect of Certain Possible Metabolic Intermediates of Butter Yellow on Rat Heart Muscle Transaminase

Compound	Final molar concentration	Inhibition, per cent
Quinone	3.5×10^{-2}	100
"	3.5×10^{-3}	66
"	3.5×10^{-4}	28
"	3.5×10^{-5}	0
N-Methyl-*p*-phenylenediamine	3.5×10^{-2}	100
"	1.4×10^{-2}	55
"	1.0×10^{-3}	0
N,N-Dimethyl-*p*-phenylenediamine	3.5×10^{-2}	100
"	1.4×10^{-2}	43
"	1.0×10^{-3}	0
p-Phenylenediamine	3.5×10^{-2}	100
"	1.4×10^{-2}	20
"	1.0×10^{-3}	0

Enzyme incubated aerobically at 38° for 15 minutes with neutralized compounds. Molar concentration refers to incubation period previous to substrate addition. Substrates: *l*(+)-glutamic acid plus oxalacetic acid.

N-methyl-*p*-phenylenediamine, N,N-dimethyl-*p*-phenylenediamine, and *p*-phenylenediamine are less active in that order. It is apparent that in sufficiently high concentrations (3.5×10^{-2}M), all these compounds completely inhibit. In the case of the aniline derivatives, a very small part of the inhibition at the higher concentrations is due to the catalytic decomposition of oxalacetic acid. Quinone is practically without effect on the decarboxylation of oxalacetic acid.

Kensler and his co-workers (10) have recently published a study of the effect of a large number of butter yellow split products on a yeast fermentation system. They found that the inhibitory effect of these various compounds could be correlated with the stability of the free radical of the inhibitor. The order of inhibitory potency found by these workers, using the same substances as those reported in this paper, was as follows: N,N-dimethyl-*p*-phenylenediamine, *p*-phenylenediamine, and quinone. It is suggested (10) that the inhibition by these compounds is due to a competition between the free radical and the diphosphopyridine nucleotide for an enzyme in the fermentation system, probably triosephosphate dehydrogenase, which requires free —SH groups for its activity.

The mechanism of the inhibition of transaminase by these compounds is at present under investigation. The fact that transaminase is not affected by those reagents, such as iodoacetate, which ordinarily react with sulfhydryl groups indicates that a different mechanism may be involved in the inhibition of this enzyme system.

Relationship of Body-Weight/Liver-Weight Ratio to Percentage Transamination

From previous studies (4) it appeared that rapidly growing tissues had a low transaminase content. In

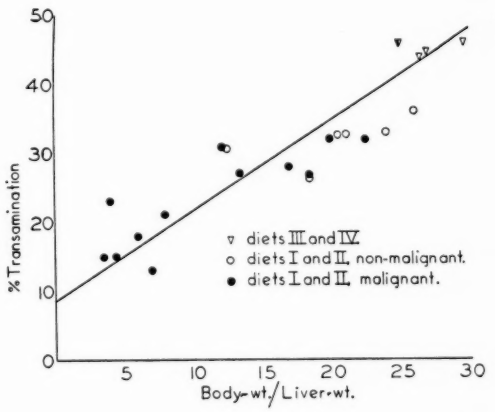

Fig. 3.—Relationship of body-weight/liver-weight ratio to percentage transamination.

view of this it seemed of interest to compare the body-weight/liver-weight ratio to the percentage transamination of animals fed butter yellow. This is shown in Fig. 3. As can be seen from the regression line derived from the experimental points, the smaller the B.W./L.W. ratio, the lower the transaminase content of the liver. Or, in other words, the more rapid the growth of the liver as compared with the general body growth, the lower is the transaminase content. The regression line is represented by the equation, $y = 9.2 + 1.27$ B.W./L.W. This means that with a decrease in the B.W./L.W. ratio of one unit, there is a percentage transamination increase of 1.27. The correlation coefficient for these data is 0.95, with an upper r value of 0.99 and a lower r value of 0.81.

The B.W./L.W. ratio of the control animals is somewhat greater than that observed for rats on an adequate diet.

Transamination in Liver from Animals Fed Butter Yellow plus Dietary Supplements

In view of the fact that butter yellow is carcinogenic only when fed together with a deficient diet, it seemed desirable to determine the transaminase activity in liver from rats fed butter yellow plus a dietary supplement. Through the kindness of Dr. Dean Burk and Dr. Charles Kensler of Cornell University, liver tissue from rats fed diet 1 plus 15 per cent yeast supplement for 200 days was made available. In addition, tumor nodules from animals not receiving the supplement were obtained. The tissues were transported in a vacuum bottle containing dry ice. Histologically the liver tissue from the animals receiving the supplemented diet showed no sign of malignancy. Transaminase activity was determined 24 hours after the death of the animal, the tissue being kept in a frozen state in the interim. The results are shown in Table III. As can be seen, the liver tissue from the animals receiving yeast supplement in addition to butter yellow shows no decrease in transaminase activity when compared with liver tissue from rats on a normal diet. This suggests that the low transaminase activity observed in butter yellow liver tumors is not due to the feeding of butter yellow *per se,* nor is it due to the inadequate diet alone (see control values in Fig. 1), but rather to the combination of both which leads to malignant changes. Undoubtedly the rats receiving an adequate diet are able to detoxify the oxidative intermediates of butter yellow and so prevent the series of changes which results in malignancy.

Table III: Transamination in Liver from Rats Fed Butter Yellow with and without 15 Per Cent Yeast Supplement

Diet supplement	Transamination, per cent
1. Butter yellow + 15 per cent yeast	53
2. " " " " " " "	55
3. Butter yellow	15
4. " "	18
5. " "	15
6. " "	13

Substrates, *l*(+)-glutamic acid plus oxalacetic acid (equimolar concentrations, 0.014). Tissue concentration, 1:80; incubation time, 15 minutes; air; temperature, 38°.

DISCUSSION

Previous to the present study, the only investigations dealing with changes in intermediary metabolism of liver during the course of butter yellow feeding are those on the glycolytic system. Thus Nakatani and others (13) reported a progressive increase in anaerobic glycolysis with continued butter yellow feeding, the highest values being realized with the resulting tumor tissue. Aerobic glycolysis, on the other hand, was found to remain at a normal low level up to the stage of tumor development, at which time higher values were observed. Orr and Stickland (15) investigated the same problem and observed that the anaerobic glycolysis values of normal liver varied over a wide range, and were found to be dependent on the glycogen content of the liver. In contrast to the findings of Nakatani and his co-workers (13), they could find no evidence supporting the claim that there was a change in glycolytic metabolism in the precancerous phase of butter yellow feeding. However, they did find that there was a qualitative change at the time of cancer formation, in that the malignant tissue was found to be able to glycolyze glucose (glucolysis) both aerobically and anaerobically in contrast to the normal and cirrhotic tissues which were able to glycolyze only glycogen. Burk and his co-workers (1), who carried out a similar investigation, found that while the anaerobic and aerobic glycolysis values increased abruptly at the time of tumor formation, there was only a small, though definite, increase in anaerobic glycolysis in the precancerous stage. No changes in Q_{O_2} values were observed by any of these groups of workers.

On the other hand, the activity of many enzyme systems and the content of other components of butter yellow liver tumors have been compared with normal liver. Thus, Greenstein and his co-workers (7–9) have reported that the ratios of enzyme activity of transplanted butter yellow liver tumor to that of normal liver are as follows: catalase, 1:1,000; arginase, 1:15; thymonucleodepolymerase, 1:1.1; xanthine dehydrogenase, 1:2; and amylase, 1:1. In addition to these systems, unpublished studies by one of us (P. P. C.) have revealed a marked decrease in activity in butter yellow liver tumors of the following systems: glyoxalase, urea synthesis, and fatty acid oxidase. In contrast to these findings, preliminary experiments indicate a slight increase in *d*-amino acid oxidase activity. The changes in activity of these different enzyme systems during the course of butter yellow feeding are at present under investigation.

It is to be noted that aside from amylase, and possibly *d*-amino acid oxidase, all the enzymes studied show a decreased activity in liver tumor as compared with normal liver.[3] Since it has been shown in this paper that a decrease in transaminase activity precedes malignant changes, it would seem highly desirable to know whether the activities of these different sys-

[3] Maver, Mider, Johnson, and Thompson have recently presented evidence that the proteinase and leucyl peptidase activity of hepatoma was greater than that of normal tissue (J. Nat. Cancer Inst., **2**:277-282. 1941).

tems also show a decrease in activity previous to carcinogenesis, or coincident with it, as seems to be the case with the glycolytic system. With such information at hand it might be possible to correlate the initiation of malignant change with quantitative variations of certain key enzyme systems.

The decrease in the activities of these various systems (with the exception of amylase and possibly *d*-amino acid oxidase) is of considerable interest, inasmuch as there is no decrease in the total amount of protein in tumor tissues. Actually the rate of protein synthesis is more rapid. Consequently one must assume that the toxic metabolic intermediates of butter yellow either specifically inhibit the synthesis of these special proteins, or that they selectively inactivate them as soon as they are formed. The very fact that only certain proteins from amongst a large number are affected by this carcinogenic agent is important in considering the possible mechanism or mechanisms of carcinogenesis.

SUMMARY

1. The transaminase activity of liver from rats fed butter yellow decreases to about one-third the initial value in the resulting tumor tissue. There is a high correlation between the days of butter yellow feeding and the transaminase activity.

2. Livers from rats fed 15 per cent yeast supplement in addition to butter yellow show normal values for transaminase activity.

3. The following possible metabolic intermediates of butter yellow, in the order of their potency, inhibit transaminase: quinone, N-methyl-*p*-phenylenediamine, N,N-dimethyl-*p*-phenylenediamine, and *p*-phenylenediamine.

REFERENCES

1. Burk, D., Behrens, O. K., and Sugiura, K. Metabolism of Butter Yellow Rat Liver Cancers. Cancer Research, Proc. Am. A. Cancer Research, **1**:733-734. 1941.
2. Cohen, P. P. Transamination with Purified Enzyme Preparations (Transaminase). J. Biol. Chem., **136**:565-584. 1940.
3. Cohen, P. P. Kinetics of Transaminase Activity. J. Biol. Chem., **136**:585-601. 1940.
4. Cohen, P. P., and Hekhuis, G. L. Transamination in Tumors, Fetal Tissues, and Regenerating Liver. Cancer Research, **1**:620-626. 1941.
5. Cohen, P. P., and Hekhuis, G. L. Rate of Transamination in Normal Tissues. J. Biol. Chem., **140**:711-724. 1941.
6. Fisher, R. A. Statistical Methods for Research Workers. Sixth edition. Pp. 339. Edinburgh: Oliver and Boyd. 1936.
7. Greenstein, J. P., and Jenrette, W. V. The Depolymerization of Thymonucleic Acid by an Enzyme System in Normal and Cancerous Hepatic and Mammary Tissues and in the Milk and Sera of Several Species. J. Nat. Cancer Inst., **1**:845-863. 1941.
8. Greenstein, J. P., Jenrette, W. V., Mider, G. B., and White, J. Chemical Studies on the Components of Normal and Neoplastic Tissues. V. The Relative Arginase Activity of Certain Tumors and Normal Control Tissues. J. Nat. Cancer Inst., **1**:687-706. 1941.
9. Greenstein, J. P., Jenrette, W. V., and White, J. The Relative Activity of Xanthine Dehydrogenase, Catalase, and Amylase in Normal and Cancerous Hepatic Tissues of the Rat. J. Nat. Cancer Inst., **2**:17-22. 1941.
10. Kensler, C. J., Dexter, S. O., and Rhoads, C. P. The Inhibition of a Diphosphopyridine Nucleotide System by Split Products of Dimethylaminoazobenzene. Cancer Research, **2**:1-10. 1942.
11. Kinosita, R. Studies on the Cancerogenic Chemical Substances. Tr. Soc. path. jap., **27**:665-727. 1937.
12. Krebs, H. A. Microdetermination of α-Ketoglutaric Acid. Biochem. J., **32**:108-112. 1938.
13. Nakatani, M., Nakano, K., and Ohara, Y. Untersuchung über den Gewebsstoffwechsel beim Verlauf der Leberkrebsentstehung durch Dimethylaminoazobenzolfütterung. Gann, **32**:240-244. 1938.
14. Orr, J. W. The Histology of the Rat's Liver during the Course of Carcinogenesis by Butter Yellow (*p*-Dimethylaminoazobenzene). J. Path. & Bact., **50**:393-408. 1940.
15. Orr, J. W., and Stickland, L. H. The Metabolism of Rat Liver during Carcinogenesis by Butter Yellow. Biochem. J., **35**:479-487. 1941.
16. Sugiura, K., and Rhoads, C. P. Experimental Liver Cancer in Rats and Its Inhibition by Rice-Bran Extract, Yeast, and Yeast Extract. Cancer Research, **1**:3-16. 1941.

Metabolic Studies in Mouse Leukemia

I. The Metabolism of Lymph Nodes in Lymphoid Leukemia*

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The identification, isolation, and study in this laboratory of various types of leukemia in mice has afforded unusual opportunity for investigation of the metabolism of cells and organs in this disease. The essential similarity of the leukemias of mice and man has been emphasized, and it has been concluded that in both species the disease is neoplastic (7). It therefore became of interest to determine whether the tissue metabolism in mouse leukemias manifested disturbances which Warburg (16) regarded as characteristic of malignant tissues. Victor and co-workers (9–15) have found in the lymph nodes of mice with spontaneous and transmitted lymphoid leukemia consistent increases in both aerobic and anaerobic glycolyses over the values obtained with normal nodes. In this paper we present the results of similar metabolic studies on spontaneous and transmitted lymphoid leukemias of mice available in this laboratory.

MATERIAL AND METHODS

Tissues of leukemic mice of two stocks have been studied. In one of these (Ak) approximately 70 per cent of the adult animals develop the disease spontaneously. The second stock (RAk) consists of different hybrids of mice of stock Ak with mice of stock R in which leukemia is rare. The tissues of two strains of transmitted lymphoid leukemia have also been studied. One of these originated in an animal of stock RAk and the other in an animal of stock Ak. Both were transmitted in mice of stock Ak.

The mice were killed by crushing the skull, no anesthetic being used. The organs to be studied were excised immediately, placed in Ringer's solution, and there cut into small pieces. The pieces were of such thickness that the rates of diffusion of oxygen, carbon dioxide, or glucose between the medium and the tissue interior were not, to our knowledge, limiting factors in the metabolic processes. The pieces were placed on blotting paper for a few seconds to remove adhering water, weighed on cover slips, and transferred as quickly as feasible to the metabolism vessels. From 60 to 200 mgm. of this moist tissue were used in each vessel.

Tissue metabolism determinations were made by the conventional manometric method of Warburg as described by Dixon (5). Oxygen consumption was measured in Ringer's solution containing M/150 phosphate buffer at pH 7.4, in a vessel with an alkali well to remove carbon dioxide from the gas phase. Carbon dioxide production was determined on tissue in the same solution in two vessels without alkali in wells. The carbon dioxide contents of the tissues and solution were obtained in one vessel at the beginning of the period of observation and in another vessel at the end of the period of observation by displacement with 3N HCl added from a sealed-in sidearm. The difference between these values was added to the carbon dioxide found in the gaseous phase, the latter being calculated from the manometer excursion in the second vessel corrected for oxygen consumption, obtained by measurement as indicated above, and calculated for an equal weight of tissue. Aerobic glycolysis was measured manometrically in Ringer's solution containing 0.025 M sodium bicarbonate equilibrated with 95 per cent O_2 + 5 per cent CO_2. The acid production was calculated from the manometer excursion corrected for both the oxygen consumption and the respiratory carbon dioxide production, measured as indicated above. Anaerobic glycolysis was measured in the same solution equilibrated with 95 per cent N_2 + 5 per cent CO_2, which had been passed over hot copper. All tissue media contained 200 mgm. per cent glucose.

Respiratory quotients were obtained in a relatively small number of experiments. The means (and their standard errors) for the various groups of experiments are shown in the following table, made up from data given in Table I.

Source of lymph nodes	Number of observations	R. Q.
Normal		
Stock Ak	5	0.87 ± 0.04
Spontaneous leukemia		
Stock Ak	11	0.86 ± 0.04
Stock RAk	4	0.97 ± 0.04
Transmitted leukemia		
Stock Ak	4	0.82 ± 0.09

* These investigations were aided by grants from The Lady Tata Memorial Trust, The International Cancer Research Foundation, and The Jane Coffin Childs Memorial Fund for Medical Research.

TABLE I: DETAILED DATA ON THE METABOLISM OF NORMAL AND LEUKEMIC LYMPH NODES

Mouse number	Sex	Age, days	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	R. Q.	Wet-weight: dry-weight ratio
			NORMAL MICE OF STOCK AK				
25	F	..	6.49	...	...	...	...
54	F	..	5.39	...	...	...	...
Ak555	F	..	...	1.99	4.81	...	...
Ak583	M	63	5.60	...	...	0.82	...
Ak584	M	55	7.11	...	...	0.95	...
Ak585	M	55	7.17	...	...	0.78	...
Ak587	M	49	7.43	...	...	0.92	...
Ak588	M	49	6.22	...	...	0.86	...
Ak617A	M	70	...	2.04	3.50	...	...
Ak617B	M	70	...	2.04	...	...	...
Ak625A	M	74	...	3.71	...	...	...
Ak625B	M	74	...	...	2.14	...	...
Ak625C	M	74	...	2.20	...	...	...
Ak625D	M	74	...	...	4.50	...	...
Ak625E	M	74	...	1.26	...	...	...
Ak625F	M	74	...	...	5.65	...	...
Ak626A	M	41	...	2.30	...	...	...
Ak626B	M	41	...	...	6.22	...	...
Ak626C	M	41	...	1.88	...	...	...
Ak626D	M	41	...	...	6.33	...	...
Ak626E	M	41	...	1.26	...	...	...
Ak626F	M	41	...	...	5.28	...	...
Ak645	F	88	...	...	2.20	...	...
Ak633A	M	50	...	...	6.90	...	...
Ak670A	M	32	5.18	...	...	...	...
Ak670B	M	32	6.69	...	...	...	...
Ak670C	M	32	...	...	1.94	...	...
Ak670D	M	32	5.75	...	...	...	...
Means			6.28	2.04	4.71	0.87	...
			SPONTANEOUS LYMPHOID LEUKEMIA IN STOCK AK				
SUBGROUP A							
Aki81	M	171	6.17	2.62	11.04	...	...
Aki114	F	258	5.96	2.56	16.32	...	5.37
Akf56	F	321	7.69	3.35	12.08	...	...
Means			6.69	2.82	13.13	...	...
SUBGROUP B							
Akh170	F	172	7.64	1.46	16.16	...	4.65
Akf124	M	215	6.01	...	...	0.86	...
Akf67	F	218	5.65	3.56	10.72	...	...
Akh145	F	232	6.28	...	...	0.85	...
Aki77	F	233	...	...	10.72	...	5.47
Akh96	M	243	7.90	...	...	...	4.22
Akh118	M	244	...	...	14.23	...	...
Akh112	F	247	8.47	2.20	13.28	...	...
Akh92	M	269	...	...	12.92	...	4.92
Akg127	F	299	6.54	2.67	13.23	0.85	...
Akg122	M	319	6.54	...	...	...	4.89
Akh83	F	401	5.07	...	...	0.79	5.33
Akg86	F	406	7.74	4.34	10.09	...	5.62
Akh76	F	423	6.49	3.29	14.70	...	5.22
Akg123	M	495	6.28	2.93	10.41	...	6.09
Means			6.80	3.09	12.34	0.84	5.16

TABLE I—*Continued*

Mouse number	Sex	Age, days	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	R. Q.	Wet-weight: dry-weight ratio
SPONTANEOUS LYMPHOID LEUKEMIA IN STOCK AK							
SUBGROUP C							
Akh146	F	232	4.76	...	...	1.02	...
Akf62	F	234	5.23	...	...	...	5.44
Aki115	F	256	4.60	4.34	11.51	...	5.95
Akf108	F	262	6.38	...	6.85	...	5.80
Akf61	F	294	5.75	...	...	0.67	4.34
Aki87	F	325	4.03	2.62	8.84	0.90	5.96
Akf74	F	331	7.85	4.76	8.79	0.95	4.74
Akg136	M	371	8.37	3.66	13.08	0.93	...
Akg110	F	389	6.54	4.76	9.05	...	5.04
Akh84	F	459	...	...	4.86	...	5.12
Means			6.07	4.03	9.00	0.89	5.29
UNCLASSIFIED							
Akh169	F	214	4.34	1.94	8.89	0.85	...
Akf174	F	225	...	0.89	9.52	...	...
Akh244	M	263	4.24	2.46	11.45	...	...
Aki120	M	282	4.97	2.72	6.80	...	...
Akh105	F	312	4.08	...	...	1.02	...
Akh292	F	335	3.35	3.87	13.75	...	...
Akh225	M	348	5.81	0.31	5.75	...	...
Akf187	F	352	5.13	3.24	5.28	...	...
Akh213	F	365	5.18	0.16	3.61	...	...
Akh193	M	370	3.92	3.97	6.12	...	...
Aki188	M	435	4.18	2.77	7.16	...	...
Aki105	F	444	...	2.77	8.94	...	...
Akf85	M	484	5.91	1.46	10.36	...	...
SPONTANEOUS LYMPHOID LEUKEMIA IN STOCK RAK							
"12.5 PER CENT AK"							
He679	F	279	4.29	0.47	8.26	...	...
"25 PER CENT AK"							
Ha185	F	665	5.44	4.71	9.52	...	...
"37.5 PER CENT AK"							
Hf878	F	307	6.01	2.09	6.64	...	...
"50 PER CENT AK"							
Hf982	M	243	...	—0.52	6.49	...	...
Hf921	F	277	6.38	0.16	5.02	...	...
Ha273	F	284	8.21	4.18	10.36	...	...
He390	M	288	6.33	...	7.37	...	...
Hh42	F	306	7.43	3.97	8.99	...	...
He391	M	338	4.65	2.77	10.09	...	...
He381	M	380	7.69	1.67	9.78	...	...
Hb78	F	385	7.01	—0.26	20.40	...	5.58
Ha964	F	432	8.68	3.35	8.47	...	...
Ha56	M	605	5.02	0.31	6.22	...	...
Hb28	F	732	6.85	3.24	9.05	...	...
Ha21	F	819	...	3.09	6.01	...	...
Means			6.83	1.99	9.02	...	...

TABLE I—*Continued*

Mouse number	Sex	Age, days	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	R. Q.	Wet-weight: dry-weight ratio
SPONTANEOUS LYMPHOID LEUKEMIA IN STOCK RAK							
"62.5 PER CENT AK"							
Hg104	F	267	7.32	0.68	8.42	...	...
"75 PER CENT AK"							
Hh358	F	139	6.54	1.94	10.83	...	...
Hb189	F	185	6.28	0.94	13.70	...	5.29
Hg484	F	187	5.86	1.36	7.48	...	...
Hg502	M	197	7.11	0.52	8.79	...	...
Hc849	F	231	5.60	0.00	7.22	...	...
Hc882	F	236	6.49	—0.37	8.32	...	...
Hc693	F	238	6.07	—0.05	7.27	...	...
Hg123	F	249	7.74	0.99	11.98	...	...
Hh406	F	255	5.86	1.52	11.66	...	...
Hf922	F	256	6.33	—0.78	...	...	...
Hf965	F	271	6.49	1.36	8.79	...	...
Ha95	F	280	...	—0.68	2.72	...	...
Hc445	F	289	6.17	—0.42	8.37	...	...
Hc449	M	293	7.37	—1.26	6.54	...	...
Ha254	M	296	4.18	3.61	11.04	...	...
Ha230	F	302	5.18	3.61	7.90	0.98	5.59
Hb170	F	304	7.64	2.14	9.26	1.04	...
Hc951	F	316	5.54	3.19	7.06	...	...
Hb193	M	320	8.84	4.71	10.56	...	...
Ha150	M	326	6.54	...	...	0.96	5.41
Ha147	M	357	7.01	3.29	6.80	...	5.21
Ha149	M	386	4.97	...	...	0.88	...
Means			6.37	1.28	7.92	...	...
"87.5 PER CENT AK"							
Hb630	F	202	5.96	1.73	11.30	...	...
Hf925	F	223	7.11	—0.10	7.01	...	...
Hf449	F	278	...	0.31	10.67	...	...
Hf836	M	286	6.01	1.05	7.27	...	...
Hf835	F	294	7.27	—0.94	6.59	...	...
Hf561	M	303	6.43	—0.16	5.91	...	...
Hc27	F	325	5.02	0.94	7.17	...	...
Hc28	F	325	...	0.31	6.22	...	...
Hf235	F	345	5.65	...	5.70	...	...
Hb658	F	374	...	...	9.68	...	...
Ha832	M	378	6.28	—0.57	6.49	...	...
Hf555	F	383	5.49	1.46	6.49	...	...
Ha668	F	493	...	1.10	8.21	...	...
Means			6.14	0.47	7.59	...	...
TRANSMITTED LEUKEMIA, LINE ARISING IN AND TRANSMITTED ON STOCK AK							
M773	..	...	6.64	...	...	0.65	...
M772	..	...	5.28	...	...	0.73	..
M778	..	...	5.02	...	...	0.85	.
M775	..	...	4.24	...	...	...	.
M779	..	...	3.40	...	...	...	..
Ak782	..	...	5.39	...	...	...	...
Ak779	..	...	6.33	2.67	6.49	...	...
Ak777	..	...	6.54	2.41	6.69	...	..
Ak778	..	...	5.13	1.73	5.96	...	. .
M744	..	...	6.64	3.56	11.24	...	..
M753	..	...	7.06	...	...	1.05	. .
M749	..	...	6.43	3.56	8.68	...	...
M736	..	...	7.01	5.13	6.49	...	...
Means			5.75	3.19	7.58	0.82	...

TABLE I—*Concluded*

Mouse number	Sex	Age, days	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	R. Q.	Wet-weight: dry-weight ratio
TRANSMITTED LEUKEMIA, LINE ARISING IN STOCK RAK, BUT TRANSMITTED ON STOCK AK *							
4Ak	..	...	6.01	..	...	...	...
5Ak	..	...	6.64	...	...	...	...
9Ak	..	...	5.75	...	11.72	...	...
12Ak	..	...	5.39	...	10.36	...	...
14Ak	..	...	5.23	2.04	7.64	...	...
15Ak	..	...	4.86	1.73	10.62	...	...
16Ak	..	...	5.81	1.52	11.19	...	...
18Ak	..	...	6.59	2.09	11.77	...	...
20Ak	..	...	6.43	1.73	13.08	...	...
24Ak	..	...	6.07	1.99	13.75	...	...
33Ak	..	...	6.75	1.94	7.58	...	...
34Ak	..	...	5.86	1.88	8.73	...	...
35Ak	..	...	6.85	1.26	8.94	...	...
47Ak	..	...	7.90	...	...	...	...
58Ak	..	...	6.22	...	...	...	...
Means			6.28	2.51	10.36	...	...

* R. Q. assumed to be 1.00 for calculation of $Q_G^{O_2}$.

Although the number of observations is rather small and the variability considerable, the quotients are for the most part well below unity and have therefore been used in the calculation of the aerobic glycolysis rates, since whether the R. Q. is assumed to be 1 or 0.1 to 0.2 less than unity makes a very noticeable difference in the final calculated values, as is evident from the formula used for calculating glycolysis:

$$Q_G^{O_2} = \frac{hk_{CO_2}}{W} - Q_{O_2}\left(\frac{k_{CO_2}}{k_{O_2}} - \text{R. Q.}\right),$$

in which $Q_G^{O_2}$ is the aerobic glycolysis rate, $-Q_{O_2}$ the oxygen consumption rate, h the manometer excursion per hour, W the weight of tissue (in mgm.), and k_{CO_2} and k_{O_2} the vessel constants for carbon dioxide and oxygen respectively. The mean R. Q. of each group of animals was used in calculating all the aerobic glycolysis rates for that group.

The bath temperature in all cases was 37.6° C.; the rate of shaking, 120 cycles per minute. The values used herein were computed on the basis of the first hour's excursions after the preliminary period of equilibration.

In many cases, portions of the tissues studied were dried and the wet-weight:dry-weight ratios calculated. The mean value of these ratios was 5.23. The metabolic activity rates were first calculated on the wet-weight basis and then multiplied by 5.23 for conversion to the conventional dry-weight basis to facilitate comparison with the data of other workers. The symbols used are those recommended by Dixon.

RESULTS

In Table I the original data are given in detail, classified by groups of animals. It will be noted that often, due to a variety of causes, such as inadequacy of the amount of material available, only one or two of the conventional three measures of metabolic activity were made on tissues from one mouse. The mean values (with their standard errors) for oxygen consumption (Q_{O_2}), aerobic glycolysis rate ($Q_G^{O_2}$), and anaerobic glycolysis rate ($Q_G^{N_2}$) of the various groups of normal and leukemic lymph nodes studied appear in Table II.

NORMAL NODES FROM MICE OF STOCK AK

Since in this stock of mice the adult animals develop leukemia in a high proportion of cases, it was deemed advisable to use mice young enough to avoid inclusion in the normal group of mice with unrecognized leukemia. The age of the mice used varied from 32 to 88 days, the mean being 57 days.

TABLE II: TISSUE METABOLISM OF NORMAL AND LEUKEMIC NODES

Source of material	Number of mice	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$
Normal				
Stock Ak	10	6.28 ± 0.26	2.04 ± 0.26	4.71 ± 0.58
Spontaneous leukemia				
Stock Ak	39	6.01 ± 0.26	3.56 ± 0.21	10.51 ± 0.68
Stock RAk	51	6.38 ± 0.16	1.31 ± 0.26	8.47 ± 0.52
Transmitted leukemia *				
Stock Ak	13	5.75 ± 0.31	3.19 ± 0.84	7.58 ± 0.84
Stock RAk	15	6.28 ± 0.16	2.51 ± 0.10	10.36 ± 0.58

* The malignant cell lines arose in the stocks indicated; both were transmitted in mice of stock Ak.

The metabolism of normal lymph nodes of mice has been studied by Victor and Potter (10). In their strain C58 at an age of 6 to 8 weeks, the mean values were: Q_{O_2}, 5.45; $Q_G^{O_2}$, 2.13, subsequently corrected to 1.0 (14); and $Q_G^{N_2}$, 5.78, subsequently stated to be about 1.4 units too high (15). The differences between their results and ours are of the order of magnitude, which, in their experience and ours, may well occur between such measurements made on different strains of normal mice.

Histological examination of typical nodes from mice of stock Ak yielded the following estimate of cellular composition: lymphocytes, chiefly small, occasionally medium-sized or large, 75 to 85 per cent; erythrocytes, endothelial cells, fibroblasts, and fibers, including capsular elements, 15 to 25 per cent. The metabolic values obtained on lymph nodes are thus not characteristic of the small lymphocytes, but of all the tissues comprising the nodes tested.

Nodes from Leukemic Mice of Stock Ak (Spontaneous Lymphoid Leukemia)

The oxygen consumption of the lymph nodes from leukemic mice of stock Ak is almost identical with that of normal mice of the same stock. However, the rates of both aerobic and anaerobic glycolysis are increased; in both instances the chances are less than 1 in 100 that the difference between the means of the leukemic and normal animals could occur in random sampling (6).

Table III: Sex and Metabolic Activity of Leukemic Lymph Nodes

	Stock Ak			Stock RAk		
	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$
Males	6.59	2.62	11.40	6.54	1.31	7.85
Females	6.07	2.51	10.98	6.49	1.26	8.58

As is shown in Table III, the sex of the animals had no significant effect on the metabolic activities of the leukemic nodes investigated.

The age of the leukemic animals had no influence on the oxygen consumption of the Ak lymph nodes; however, the glycolysis rates are affected to some degree. As may be seen in Table IV, the aerobic glycolysis rate rises progressively with age up to at least 400 days; in this age range the Pearsonian coefficient of correlation (r) between these measures is $+0.65$ with a standard error of 0.14. In the oldest group, with ages between 400 and 550 days, the aerobic glycolysis rate appears to fall below the maximum. In view of the small number of determinations involved (six) and of the fact that in the RAk strain the increase in $Q_G^{O_2}$ with age persists to the fourth age group, it may well be that the value of 2.98 for the Ak strain is definitely low; the difference between the oldest group and that of ages between 300 and 399 days could occur about once in 20 times by chance. The anaerobic glycolysis rate, however, definitely tends to fall with increasing age; the correlation coefficient is -0.50 with a standard error of 0.33; this correlation is considerably less significant than that of age with aerobic glycolysis rate.

The increase in aerobic glycolysis rate with age in our leukemic animals is probably rendered more significant by the observation of Victor and Potter (10) that, in four strains of normal mice, the aerobic glycolysis rate of the lymph nodes decreases with age. Consequently the difference between the leukemic and normal nodes will increase progressively with age. Some light on the mechanism involved may be gained from the finding of Victor and Potter (12) that, when malignant lymphocytes of one line are injected into young and old mice of a single stock, the aerobic glycolysis rate of the resulting leukemic lymph nodes is lower in the older than in the younger host animals. This finding, together with that of the decline in this metabolic process with age in normal animals, shows that there is a host factor in the older animals decreasing the aerobic glycolysis rate of lymphocytes. If this conclusion be applicable to our stock of mice, then the rise in aerobic glycolysis rate observed in our leukemic animals would appear to be even more clearly indicated and to represent an expression of differences in the glycolytic activities of the lymphocytes becoming malignant at different ages of the host.

An entirely different situation exists in the relation between the rate of anaerobic glycolysis and age. The negative correlation observed in our leukemic animals is paralleled by a similar correlation in the

Table IV: Influence of Age on the Metabolism of Leukemic Lymph Nodes

Age range, days	Stock Ak				Stock RAk			
	Number of mice	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	Number of mice	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$
125–224	5	5.65 ± 0.07	2.62 ± 0.53	11.72 ± 1.78	6	6.38 ± 0.26	1.05 ± 0.31	9.83 ± 1.10
225–299	12	5.91 ± 0.37	2.93 ± 0.37	11.51 ± 1.05	19	6.38 ± 0.26	1.16 ± 0.31	8.42 ± 0.47
300–399	13	5.54 ± 0.37	3.87 ± 0.26	8.42 ± 1.05	16	6.33 ± 0.26	1.88 ± 0.31	7.85 ± 0.42
400–550	6	5.96 ± 0.53	2.98 ± 0.37	9.52 ± 1.26	6	6.49 ± 0.84	2.62 ± 0.68	7.58 ± 0.94

The values given are means with standard errors of means.

normal mice of Victor and Potter (10). Further, in the transmission experiments referred to in the preceding paragraph, these workers found that the anaerobic glycolysis rate of the lymph nodes of the older leukemic animals was lower than that in the younger. These considerations suggest that in both leukemic and normal animals a host factor depressing the rate of anaerobic glycolysis appears or is intensified with advancing age.

Attention should be called to the fact that our leukemic animals are considerably older than our normal controls. Victor and Potter (10) found in four different strains of normal mice that both aerobic and anaerobic glycolysis rates of lymph nodes declined with age. The increases we observed in the malignant lymph nodes as compared with our controls would thus have probably been still greater had they been compared with controls of the same host age.

The mean values (with their standard errors) for the metabolic activities studied in each subgroup are given in Table V. The oxygen consumption rate of subgroup C is lower than that of subgroup B by an amount which could occur about once in 4 times; the difference is thus not significant. A factor operating to reduce the oxygen consumption of subgroup C is the presence of more intercellular fluid, evidenced microscopically and also possibly by the slightly higher wet-weight:dry-weight ratio in subgroup C as compared with subgroup B.

The aerobic glycolysis rate increases moderately but progressively from subgroup A to subgroup C. The difference between the means of subgroups B and C is one which would occur about once in 50 times in random sampling. The anaerobic glycolysis rate, conversely, decreases progressively from subgroup A to subgroup C. The difference between the means from subgroup B and C is one which would occur less than once in 100 times in random sampling.

TABLE V: METABOLISM OF LYMPH NODES OF MICE OF SUBGROUPS OF STOCK AK WITH SPONTANEOUS LEUKEMIA

Subgroup	Estimated proportion of malignant cells, per cent	Number of mice	Observed metabolic values			Estimated metabolic values for malignant cells		Wet-weight: dry-weight values
			Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$	$Q_G^{O_2}$	$Q_G^{N_2}$	
A	95–100	3	6.69 ± 0.68	2.82 ± 0.42	13.13 ± 1.99	3.40	13.44	..
B	90–95	15	6.80 ± 0.26	3.09 ± 0.37	12.34 ± 0.68	3.71	12.24	5.16
C	70–90	10	6.07 ± 0.52	4.03 ± 0.47	9.00 ± 1.10	4.50	10.04	5.29

The values given for observations are the means with their standard errors.

CORRELATION WITH MORPHOLOGICAL CHARACTERISTICS

Degree of leukemic infiltration.—Histological examination of touch preparations and sections prepared from the leukemic nodes studied showed that there was considerable variation in their cellular composition, particularly in the proportion of malignant lymphocytes to other components of the normal node (small lymphocytes, fibroblasts, endothelial cells, etc.). On this basis the leukemic nodes from mice of stock Ak were divided into three subgroups: (a) pure (approximately 95 per cent or more of the organ bulk composed of malignant cells); (b) good (approximately 90 to 95 per cent); and (c) relatively impure (less than approximately 90 per cent of bulk composed of malignant cells).

No significant metabolic differences could be discovered to be related to the different types of materials (small lymphocytes, histiocytes, cellular debris, red corpuscles, and framework cells) that were found in the lymph nodes in addition to malignant lymphocytes.

Since the distribution of animals into the subgroups according to age as tested by application of the χ^2 test (6) did not depart significantly from that which would result from chance, there was no evidence that age was a factor in determining the degree of leukemic infiltration.

We have attempted to estimate in each subgroup the aerobic and anaerobic glycolysis rates of the malignant lymphocytes themselves, basing the calculations upon the mean rates obtained for the lymph nodes, the estimated proportion of malignant to total tissue bulk, and the assumption that the nonmalignant elements have the same glycolytic rates as normal lymph nodes. The results of these calculations appear in Table V.

The progressive changes in the glycolytic rates among the subgroups must be due either to inherent differences in the glycolytic capacities of the malignant lymphocytes in the subgroups or to factors arising from metabolic interaction of malignant and nonmalignant elements in the tissues studied. With regard to the former alternative, no cytological differences were observed between the malignant lymphocytes of the various subgroups. With regard to the latter alternative, a finding of Victor and Potter (13) may be cited. These workers found that, following the intraperitoneal injection of malignant lymphocytes into susceptible mice, the anaerobic glycolysis rate of normal (uninfiltrated) lymph nodes is reduced below the control level, presumably because

of some humoral agent arising either from the malignant cells or the interaction of such cells with the host organism. In our subgroup C, which has the largest proportion of normal elements, the observed low rate of anaerobic glycolysis might be due, at least in part, to a similar influence of the malignant cells upon the normal elements. However, even if it is assumed that the anaerobic glycolysis rate of the normal cells is reduced to zero, that of the malignant cells would be 11.25, a value which is still below that of the malignant cells of the other subgroups. It follows that, under the conditions existing in tissues of subgroup C, the malignant cells themselves have a lower anaerobic glycolysis rate.

Density of infiltration of the nodes by leukemic cells.—A second histological characteristic of the leukemic nodes which could be correlated with metabolic findings was the closeness with which the leukemic cells are in contact with each other. Within any one subgroup the rate of anaerobic glycolysis increases consistently with increasing density. Although this may be in part due to changes in lymph content, the wet-weight:dry-weight ratio differences show that this influence is inadequate to explain the degree of change.

Nodes from Leukemic Mice of Stock RAk

As may be seen from Table II, the oxygen consumption values of the leukemic lymph nodes of stock RAk do not significantly differ from those of stock Ak. However, the aerobic and anaerobic glycolysis rates in stock RAk are significantly lower, the chances of the observed differences occurring by chance being less than 1 in 100. In 10 cases the aerobic glycolysis rate was zero or slightly negative. The respiratory quotients in stock RAk are apparently higher than in stock Ak, although the data for RAk are admittedly almost too meager to be generalized. Subgrouping on the basis of degree of leukemic infiltration is not justified here, since in only 2 instances were normal cells present in conspicuous numbers in the tissues studied.

As in stock Ak, the sex of the mice had no influence on the metabolic activities of the lymph nodes (see Table III). The age of the animals, however, affected the glycolytic rates of their lymph nodes, as may be seen in Table IV. The aerobic glycolysis rate increased with age, the correlation coefficient being 0.36 with a standard error of 0.13; while the anaerobic glycolysis rate decreased, the correlation coefficient, after exclusion of one aberrantly high and one low value, being −0.22 with a standard error of 0.14. These correlations are so low that, standing alone, they would have little significance. However, their significance is supported by the fact that they have the same sign as the corresponding correlations for stock Ak. The possible meaning of the effect of age in increasing the aerobic and decreasing the anaerobic glycolysis rates of leukemic lymph nodes will be discussed later in this paper.

The mice of the stock designated RAk are hybrids derived from stocks Ak (highly susceptible to leukemia) and R (resistant to leukemia); they include not only members of the F_1 generation (with "50 per cent Ak heredity"), but also F_2 backcrosses to R ("25 per cent Ak heredity") and F_3 backcrosses (giving intermediate "percentages of Ak heredity"). In Table VI the metabolic activity of the leukemic lymph nodes from animals with various percentages of Ak heredity is compared with the data from stock Ak itself. The oxygen consumption means for the groups increase progressively with decreasing "percentage of Ak heredity." The differences are small; but, since the difference between "100 per cent" and "50 per cent" Ak heredity would occur only once in 100 times by chance, the relation deserves some credence. The aerobic and anaerobic glycolysis rates show a minimum in mice with "87.5 per cent Ak heredity." The differences between "100 per cent" and "87.5 per cent Ak heredity" groups for both glycolysis rates are highly significant. The differences between "87.5 per cent" and "50 per cent Ak heredity" are much less significant, there being 2 chances in 100 for the aerobic glycolysis difference, and 30 in 100 for the anaerobic glycolysis difference, to occur at random. The statistical analysis thus gives considerable support to the credibility of this nonlinear relation of the proportion of "Ak heredity" to the rates

Table VI: Influence of Different "Percentages of Heredity" from the High Leukemia Stock (Ak) upon the Metabolism of Their Lymph Nodes

"Ak heredity," per cent	Number of mice	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$
100	39	6.01 ± 0.26	3.56 ± 0.21	10.51 ± 0.68
87.5	13	6.14 ± 0.26	0.47 ± 0.27	7.59 ± 0.53
75	22	6.37 ± 0.23	1.28 ± 0.39	7.92 ± 0.62
50	12	6.83 ± 0.43	1.99 ± 0.54	9.02 ± 1.20

The figures given are means with standard errors of means.

of aerobic and anaerobic glycolysis. The relative significance of inheritance from leukemia-susceptible and leukemia-resistant stocks is not clear.

Nodes from Mice of Stock Ak with Transmitted Leukemia Originating in Stocks Ak and RAk

The metabolic activity of leukemic lymph nodes resulting from the transfer into mice of stock Ak of two lines of malignant lymphocytes, one of which arose spontaneously in stock Ak, the other in stock RAk, has been studied. The results which appear in Table II are similar in order of magnitude to those obtained on the lymph nodes of mice which developed leukemia spontaneously. Detailed comparison of these findings with those from spontaneous leukemic nodes are scarcely necessary here, but two circumstances should be noted in any attempt to do so. First, the recipients of the transmission lines were considerably younger than the mice in which the leukemia arose. Second, we do not have metabolic data on the lymph nodes of the particular mice in which the transmission lines arose; it would probably, but not necessarily, be justified to assume that such nodes had a metabolic rate close to the average for all the lines arising in their respective stocks.

DISCUSSION

The results obtained in our studies of the metabolism of lymph nodes of mice with spontaneous leukemia are confirmatory of those of Victor and Potter (11). The means for their stock C58 are Q_{O_2}, 5.62; $Q_G^{O_2}$, 4.32 (about 3.0 corrected); and $Q_G^{N_2}$, 10.26 (about 8.8 corrected). These may be compared with our data in Table II.

The relation of the metabolic activity of tissues to the malignant state has been reviewed by Burk (1). He has traced the historical succession of the various measures of metabolism which have been regarded as useful in characterizing malignant tissues. We have calculated these measures (with a few exceptions) for our normal and leukemic lymph nodes of stock Ak. They appear in Table VII. Unlike most data on the metabolism of malignant tissues, those on malignant lymph nodes are accompanied by corresponding data on the homologous normal lymphoid tissue from animals of the same strain. Certainly still better control values would be yielded by normal lymphoid cells (lymphoblasts) of the same morphological maturity as the malignant leukemic cells; such are, however, not available in sufficient quantity for metabolic investigation.

It has been claimed not infrequently that the most consistent single difference between the metabolism of normal and malignant tissues is the higher rate of aerobic glycolysis shown by the latter, *cf.* Dickens (3). While the rate of aerobic glycolysis in our spontaneous leukemia tissues of stock Ak is significantly greater than that of the normal lymph nodes of the same stock, the absolute value remains much below that of typical malignant tumors. It has been observed by several workers, notably by Murphy and Hawkins (8), Crabtree (2), and Dickens and Šimer (4), that the rate of aerobic glycolysis of mouse tumors, in contrast with those of the rat and man, is extremely variable and tends to be relatively low (or actually zero), even when the tumors are highly malignant as judged by the rate of growth and other biological criteria. The low value obtained by us may thus be due to a species characteristic. The leukemic nodes of our stock RAk show a rate of aerobic glycolysis lower than that of the normal Ak nodes; 10 of the cases in fact had a rate of zero or slightly less. Absence of data on normal nodes of this stock renders evaluation unsatisfactory.

Table VII: Characteristics of Metabolism of Normal and Malignant Tissues

Measure of metabolism	Formula	Normal lymph nodes (stock Ak)	Nodes in lymphatic leukemia: Spontaneous, Stock Ak	Spontaneous, Stock RAk	Transmitted, Stock Ak	Transmitted, Stock RAk
Oxygen consumption	Q_{O_2}	6.28	6.01	6.38	5.75	6.28
Aerobic glycolysis	$Q_G^{O_2}$	2.04	3.56	1.31	3.19	2.51
Anaerobic glycolysis	$Q_G^{N_2}$	4.71	10.51	8.47	7.58	10.36
Absolute Pasteur effect	$Q_G^{N_2} - Q_G^{O_2}$	2.67	6.95	7.16	4.39	7.85
Per cent Pasteur effect	$\frac{100(Q_G^{N_2} - Q_G^{O_2})}{Q_G^{N_2}}$	57%	66%	85%	58%	76%
Meyerhof oxidation quotient	$\frac{Q_G^{N_2} - Q_G^{O_2}}{Q_{O_2}/3}$	1.28	3.47	3.36	2.28	3.76
Fermentation excess (U)	$Q_G^{N_2} - 2Q_{O_2}$	−7.85	−1.51	−4.29	−3.92	−2.20

The presence of active aerobic glycolysis has been attributed by Warburg at times to a decreased extent of respiration, at times to a decreased effectiveness of respiration. In our leukemic material the oxygen consumption is not significantly different from that of normal lymphoid tissue. Certainly its respiration is not defective in extent. Its effectiveness in suppressing glycolysis, *i.e.*, the Pasteur effect, may be estimated by several measures: by the absolute difference between the rates of anaerobic and aerobic glycolysis, by the percentage inhibition of anaerobic glycolysis, or by the Meyerhof oxidation quotient. As may be seen in Table VII, these measures agree in showing that the Pasteur reaction is both more extensive (absolute Pasteur effect) and more effective (Meyerhof oxidation quotient) in the leukemic than in normal lymphoid tissue. This confirms the view of Burk (1) that, contrary to much current opinion, the Pasteur reaction is not impaired in malignant tissues.

The rate of anaerobic glycolysis is definitely higher in leukemic than in normal lymph nodes. However, since the oxygen consumption is high, the fermentation excess (U) of the leukemic tissue is negative, so constituting an exception to the generalization once set forth by Warburg, though no longer held by him, that malignant tissues have positive U values. Murphy and Hawkins (8), however, found negative U values for many mouse tumors; again, as in the case of aerobic glycolysis, a species characteristic of the mouse may be involved. Thus, leukemic lymph nodes are actively glycolyzing tissues in which the Pasteur effect is considerable. Their metabolic activity is similar to that of other forms of malignant disease in the mouse.

On examination of the aerobic and anaerobic glycolysis rates of the individual leukemic animals of stock Ak, the surprising relationship appears that when one is low the other tends to be high. With the exclusion of those cases in which the Q_{O_2} was below 1.00, the correlation coefficient (r) between $Q_G^{O_2}$ and $Q_G^{N_2}$ for the remaining cases becomes -0.82, a value which could arise by chance less than once in 100 times. At least two factors tending to produce this correlation have been recognized: (a) the age of the leukemic mice, since the $Q_G^{O_2}$ tends to rise and the $Q_G^{N_2}$ to fall with advancing age; and (b) the histological characteristics of the lymph nodes, the $Q_G^{O_2}$ falling and the $Q_G^{N_2}$ rising with increasing proportions of malignant lymphocytes in the nodes. Our data are not sufficiently numerous to show whether these two factors are adequate to account fully for the observed negative correlation.

There is some evidence for the existence of a similar relationship in the animals of stock RAk with spontaneous leukemia, and in the animals with transmitted leukemia, but the data of Victor and Potter (11) on spontaneous lymphatic leukemia fail to reveal a similar tendency.

SUMMARY AND CONCLUSIONS

The oxygen consumption of the lymph nodes of mice with spontaneous and transmitted lymphoid leukemia is not significantly different from that of normal lymph nodes. The rate of aerobic glycolysis is often, but not invariably, increased; that of anaerobic glycolysis is invariably increased. The Pasteur effect is greatly increased in the leukemic as compared with the normal lymphoid tissues studied. The metabolic activity of the leukemic tissue is similar to that of malignant tumors of mice but differs from that of human and rat tumors by a lower rate of aerobic glycolysis and a slightly negative value for the fermentation excess.

In leukemic hybrids between high and low leukemia stock mice, the rate of oxygen consumption is higher and that of aerobic and anaerobic glycolysis is lower than in high leukemia stock mice. The relative significance of the percentage inheritance from leukemia-resistant and leukemia-susceptible stock remains to be determined.

In those leukemic lymph nodes which contain considerable normal tissue, the aerobic glycolysis rate of the malignant lymphocytes is higher and the anaerobic glycolysis rate is lower than in the nodes consisting almost entirely of malignant cells. With increasing age the character of the metabolism of the leukemic lymph nodes changes, the aerobic glycolysis rate increasing, the anaerobic glycolysis rate decreasing. These changes appear to be due rather to differences in the glycolytic activities of the lymph nodes themselves than to factors in the body of the host.

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REFERENCES

1. Burk, D. A Colloquial Consideration of the Pasteur and Neo-Pasteur Effects. Cold Spring Harbor Symposia on Quantitative Biology, **7**:420-455. 1939.
2. Crabtree, H. G. Observations on the Carbohydrate Metabolism of Tumours. Biochem. J., **23**:536-545. 1929.
3. Dickens, F. Metabolism of Tumours. Nature, **145**:512-513. 1940.
4. Dickens, F., and Šimer, F. The Metabolism of Normal and Tumor Tissue. II. The Respiratory Quotient, and the Relationship of Respiration to Glycolysis. Biochem. J., **24**:1301-1326. 1930.
5. Dixon, M. Manometric Methods as Applied to the Measurement of Cell Respiration and Other Processes. Cambridge: The University Press. 1934.
6. Fisher, R. A. Statistical Methods for Research Workers. Sixth edition. Edinburgh: Oliver and Boyd. 1936.

7. Furth, J., Ferris, H. W., and Reznikoff, P. Relation of Leukemia of Animals to Leukemia of Man. J. A. M. A., **105**:1824-1830. 1935.
8. Murphy, Jas. B., and Hawkins, J. A. Comparative Studies on the Metabolism of Normal and Malignant Cells. J. General Physiol., **8**:115-130. 1925.
9. Victor, J., and Potter, J. S. Studies on Mouse Leukemia. XI. Metabolic Effects of Host Constitution. J. Exper. Med., **60**:547-558. 1934.
10. Victor, J., and Potter, J. S. Studies in Mouse Leukaemia: Preleukaemic Changes in Lymphoid Metabolism. Brit. J. Exper. Path., **16**:243-252. 1935.
11. Victor, J., and Potter, J. S. Studies in Mouse Leukaemia: Metabolic Observations in Spontaneous Lymphatic Leukaemia. Brit. J. Exper. Path., **16**:253-265. 1935.
12. Victor, J., and Potter, J. S. Host Age and Cell Metabolism in Mouse Lymphatic Leukemia. Proc. Soc. Exper. Biol. & Med., **33**:609-611. 1936.
13. Victor, J., and Potter, J. S. Influence of Transmitted Leukaemia on Metabolism of Uninfiltrated Lymphoid Tissue. Brit. J. Exper. Path., **19**:227-238. 1938.
14. Victor, J., and Potter, J. S. The Respiratory Quotients of Normal and Leukemic Mouse Lymphoid Tissue. Am. J. Cancer, **32**:554-560. 1938.
15. Victor, J., and Potter, J. S. Leukemia Cell Metabolism in Serum of Normal, Immunized and Leukemic Mice. Am. J. Cancer, **33**:568-577. 1938.
16. Warburg, O. Ueber den Stoffwechsel der Tumoren. Berlin: J. Springer. 1926.

Inhibition of the Autoxidation of Aldehydes by Carcinogenic Chemicals and Related Compounds*

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Information pertinent to the mechanism of carcinogenesis by the hydrocarbons might be elicited if some relationship between biological response and chemical activity could be demonstrated; and therefore any chemical reaction of the hydrocarbons is of interest, regardless of whether any definite biological relationship can be shown. One action of the carcinogens which may be of some biological significance is the inhibition of the oxidation of phospholipids (6, 10). Other observations regarding possible biochemical reactions of the hydrocarbons have been made by Fieser (7) and by Boyland (4). Examination of certain other chemical reactions involving compounds related to the carcinogens might also prove to be of interest. One well-known reaction which easily lends itself to such study is the inhibition of the autoxidation of benzaldehyde by anthracene (1, 2, 5, 9) and other chemicals (3, 8, 13, 14, 15). The inhibiting effect of anthracene is a temporary one, involving the induced oxidation of anthracene to anthraquinone (2). Following this, the benzaldehyde autoxidizes at the usual rate. Because of the chemical relationship of anthracene to the carcinogenic hydrocarbons, it was thought desirable to test the effect of certain carcinogens on this oxidation. Furthermore, the effect of these substances on heptaldehyde was also determined, since it has been shown that this substance has some influence on the growth of certain tumors (11).

MATERIALS, METHODS, AND RESULTS

The experiments were performed in an ordinary Warburg manometric apparatus at 24° C. One-tenth milliliter samples of the aldehyde solutions were pipetted into the flasks and allowed to come to equilibrium in the bath for 10 minutes before readings were started. The following compounds were tested for inhibiting effect: anthracene, phenanthrene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene, 20-methylcholanthrene, dimethylaminoazobenzene (butter yellow), and hydroquinone. All were crystalline compounds obtained commercially. The butter yellow was recrystallized before use. Before each experiment the benzaldehyde and heptaldehyde were distilled at least 3 times under reduced pressure in an atmosphere of nitrogen in a modified Claisen flask with a fractionating column. They were of sufficient purity to show no induction period in the oxidation. From 2 to 10 runs were made with each compound at a given concentration. The results are shown in Table I. The rate of oxidation was measured at 5 minute intervals for 40 minutes, during which time the rate was fairly constant (Fig. 1).

The rate of oxidation of heptaldehyde was considerably faster than that of benzaldehyde, although the oxidation of both was rapid. Benzpyrene, dibenzanthracene, and methylcholanthrene strongly inhibited the oxidation of these aldehydes (Table I). It was noted that the carcinogens were considerably better inhibitors than anthracene, while phenanthrene was without effect. It is of interest that butter yellow, although unrelated chemically to the other substances, was also a good inhibitor. The amount of inhibition was dependent upon the amount of hydrocarbon present and varied to some extent in the separate runs.

TABLE I: THE INHIBITION OF THE OXIDATION OF ALDEHYDES

Inhibitor	Average per cent inhibition of O_2 uptake: Benzaldehyde, Concentration of inhibitor M/1,000	Benzaldehyde, M/10,000	Heptaldehyde, M/1,000	Heptaldehyde, M/10,000
Anthracene	96.4	2.2	53.3	...
Benzpyrene	99.5	97.0	96.5	20.9
Dibenzanthracene	88.5	32.6	79.5	18.0
Methylcholanthrene	99.0	22.8	96.0	6.0
Phenanthrene	0	...	...	...
Hydroquinone	...	...	18.0	14.0
Butter yellow	94.1	78.5	...	...

To determine whether the hydrocarbons were oxidized to quinones, as has been shown for anthracene (2), a solution of benzpyrene, M/100, in benzaldehyde was allowed to stand for several days to assure essentially complete oxidation of the hydrocarbon. The reaction mixture, then a dark reddish color, was diluted 1:50 with xylene, and the absorption spectrum was

* This investigation was aided by a grant from the Jonathan Bowman Fund for Cancer Research.

determined, using a Cenco spectrophotelometer. This curve was compared with that given by the mixed 5,8- and 5,10-benzpyrene-quinones obtained by the chromic acid oxidation of benzpyrene (12). The curves were almost identical except in the range of 4,000–4,300 Å, where the curve obtained from

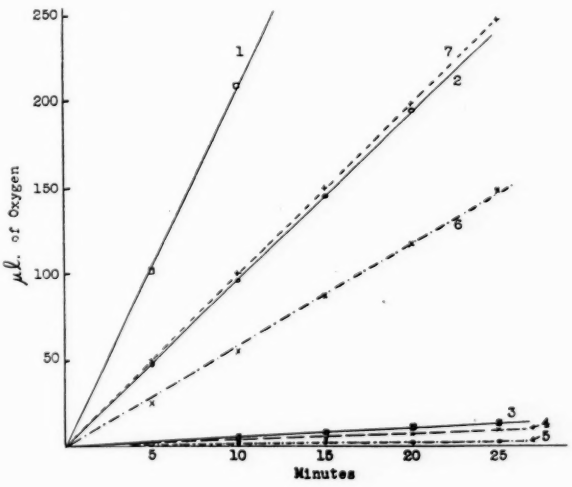

FIG. 1

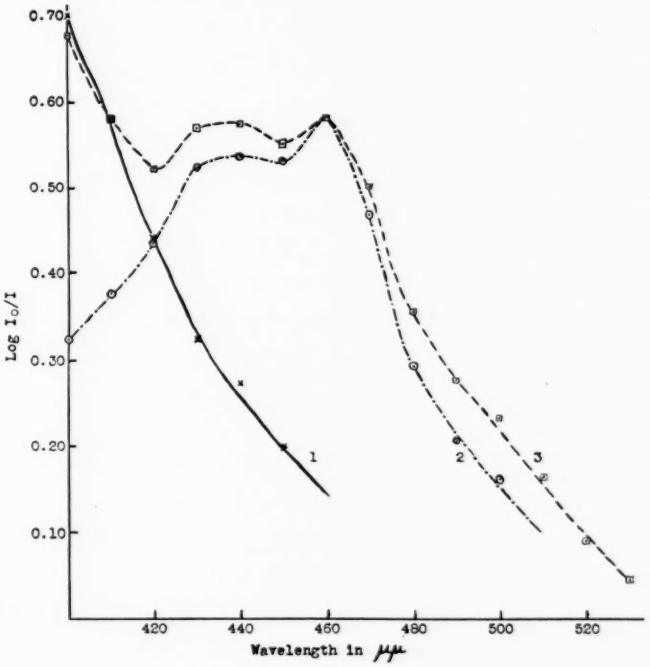

FIG. 2

FIG. 1—Rates of oxidation of benzaldehyde and of heptaldehyde in the presence of hydrocarbons, 0.1 ml. samples, 24° C.
1, Pure heptaldehyde; 2, pure benzaldehyde; 3, benzaldehyde with M/1,000 dibenzanthracene; 4, benzaldehyde with M/1,000 anthracene, or heptaldehyde with M/1,000 benzpyrene or methylcholanthrene; 5, benzaldehyde with M/1,000 benzpyrene or methylcholanthrene; 6, heptaldehyde with M/1,000 dibenzanthracene; 7, heptaldehyde with M/1,000 anthracene.

FIG. 2—Absorption spectra of benzpyrene-benzaldehyde oxidation products.
1, Absorption of old benzaldehyde; 2, benzpyrene-quinones, M/15,000 in xylene; 3, benzpyrene-benzaldehyde oxidation products, M/100, diluted 1:50 with xylene.

1,2,5,6-dibenzanthracene, 20-methylcholanthrene, dimethylaminoazobenzene, and hydroquinone.

2. Evidence from the absorption spectra indicated that the inhibition of the autoxidation of benzaldehyde by 3,4-benzpyrene involves the induced oxidation of the latter to one or more quinones.

the benzpyrene-benzaldehyde oxidation indicated the presence of a pale yellow compound. This probably resulted from products which arise in the oxidation of benzaldehyde, since it is well known that old benzaldehyde is often yellowish. Accordingly, the absorption spectrum of some old benzaldehyde, diluted with xylene, was determined, and was found to be in close agreement with that portion of the curve obtained with the benzpyrene-benzaldehyde oxidation mixture and not characteristic of the benzpyrene-quinones (Fig. 2). Since both anthracene (2) and benzpyrene are oxidized to quinones during the inhibition of the autoxidation of benzaldehyde, it is probable that the other hydrocarbons react in a similar manner.

SUMMARY

1. The autoxidation of benzaldehyde and of heptaldehyde was inhibited by anthracene, 3,4-benzpyrene,

REFERENCES

1. ALMQUIST, H. J., and BRANCH, G. E. K. The Absorption of Oxygen by Benzaldehyde. J. Am. Chem. Soc., **54**:2293-2302. 1932.
2. BÄCKSTRÖM, H. L. J., and BEATTY, H. A. The Inhibitory Action of Anthracene in the Autoxidation of Benzaldehyde. J. Phys. Chem., **35**:2530-2567. 1931.
3. BODENDORF, K. Beitrag zur Kenntnis der Inhibitor-Wirkungen. Ber. d. deutsch. chem. Gesellsch., **66B**:1608-1611. 1933.
4. BOYLAND, E., and BOYLAND, M. E. Studies in Tissue Metabolism. The Effect of Oxidized 1,2,5,6-Dibenzanthracene. Biochem. J., **28**:244-256. 1934.
5. BRANCH, G. E. K., ALMQUIST, H. J., and GOLDSWORTHY, E. C. The Induced Oxidation of Anthracene in the Autoxidation of Benzaldehyde. J. Am. Chem. Soc., **55**:4052-4062. 1933.
6. DEUTSCH, H. F., KLINE, B. E., and RUSCH, H. P. The Oxidation of Phospholipids in the Presence of Ascorbic Acid and Carcinogenic Chemicals. J. Biol. Chem., **141**:529-538. 1941.

7. Fieser, L. F. Production of Cancer by Polynuclear Hydrocarbons. University of Pennsylvania Bicentennial Conference, Philadelphia, 1941.
8. Kuhn, R., and Meyer, K. Über die Autoxydation des Benzaldehyds. Naturwissenschaften, **16**:1028-1029. 1928.
9. Milas, N. A. Auto-oxidation. Chem. Rev., **10**:295-364. 1932.
10. Rusch, H. P., and Kline, B. E. The Inhibition of Phospholipid Oxidation by Carcinogenic and Related Compounds. Cancer Research, **1**:465-472. 1941.
11. Strong, L. C. The Effect of Heptyl Aldehyde-Sodium Bisulfite on Spontaneous Tumors of the Mammary Gland in Mice. Cancer Research, **1**:473-476. 1941.
12. Vollmann, H., Becker, H., Corell, M., and Streeck, H. Beiträge zur Kenntnis des Pyrens und seiner Derivate. Liebigs Ann. d. Chem., **531**:1-159. 1937.
13. Wittig, G., and Henkel, K. Über die Autoxydation des Benzaldehyds in Gegenwart von 9,10-Diphenyl-acenaphtylen. Liebigs Ann. d. Chem., **542**:130-144. 1939.
14. Wittig, G., and Lange, W. Über die Autoxydation des Benzaldehyds in Gegenwart von Dibiphenylen-äthylen. Liebigs Ann. d. Chem., **536**:266-284. 1938.
15. Wittig, G., and Pieper, G. Über gehemmte und beschleunigte Autoxydation des Benzaldehyds in Gegenwart von Dibiphenylen-äthylen. Liebigs Ann. d. Chem., **546**: 142-171. 1941.

The Induction of Tumors with Extracts from Human Livers and Human Cancers*†

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The theory that there may be chemical causes for many human tumors in addition to those, internal and external, now known to be due to chemicals is comparatively new. The list of human external tumors which are chemically induced is quite large. It includes cancer of the skin following prolonged exposure to arsenic in industry (26) or in medication (17); cancer of the skin in gas works and other industries following exposure to coal tar (39) caused by the carcinogen 3,4-benzpyrene (6); cancer of the skin in cotton mule-spinners (32) due to the action of lubricating oil; and others. The list of human visceral tumors caused by specific chemicals is smaller. It includes, at the present time, aniline tumors of the urinary bladder (28) caused by β-naphthylamine (16); sarcomas of bone caused by radium salts (24); and some carcinomas of the lung caused by the inhalation of radioactive ores (13, 27), and possibly also by chrome compounds (38), asbestos (22, 15), and iron oxide (9).

Chemical agents responsible for human visceral tumors could conceivably be of exogenous or endogenous origin. If exogenous, the portals of entry would include the alimentary tract (17, 16), the respiratory tract (13, 22, 9), and the skin, and the chemical nature of the agents might show wide variation. If endogenous, they might be some pathological substance, or a physiological compound present in pathological amounts (21) or acting under abnormal circumstances.

The theory of chronic irritation as the cause of certain cancers was based upon the clinical observation that in man some cancers begin at points of prolonged, repeated, small traumas. Outstanding examples are the cancers which occur in the gall bladder and renal pelvis in relation to stones; cancers of the mouth in relation to broken teeth; malignant melanomas resulting from trauma, surgical or otherwise, to a pigmented mole; cancers of the lower lip in relation to pipestems; and cancers of the breast in relation to stasis of inspissated secretions. Although the original explanation, that such traumas led to cell proliferation for purposes of repair and replacement and that this cell reproduction finally went beyond the bounds of control and became irreversible, has not had experimental confirmation, the theory need not be discarded but may need modification. The basic observations can possibly be correlated with a chemical theory of the causation of cancers. The chronic irritation theory was based on the cell as the fundamental unit, whereas still more fundamental, chemical bases undoubtedly exist. That chemical explanations were not forthcoming, despite the old observations that certain substances such as tar, arsenic, paraffin, lubricating oil, etc., could cause cancers, seems strange until it is remembered that these examples of chemically induced cancer accounted for a comparatively small percentage of the cancers of one tissue only, the skin, and that they were thought to represent curiosities and not a general principle. Only with the isolation of 3,4-benzpyrene from tar in 1933 by Cook, Hewitt, and Hieger (6) did the relationship of the chemically induced group of tumors to a broad chemical theory for the causation of cancers finally appear. And with the principle established that some kinds of human cancer were caused by a chemical compound, related to substances occurring naturally in the body, it was reasonable to wonder if endogenous carcinogenic substances might be formed at or concentrated in sites of chronic injury and there eventually induce tumors. If this were shown to be true, the clinical observations of tumor formation at sites of chronic mechanical irritation might be correlated with work originating in the experimental laboratory. Furthermore the theory of chronic irritation as a cause of cancer would be correlated with a more fundamental chemical theory, which already has some proven basis.

Experimental attempts to find cancer-producing substances in relation to human cancers were the logical sequel to a number of converging lines of evidence and ideas of recent years. Some of these are:

(a) The demonstration by isolation and identification of the active cancer-producing principle in coal tar that this chemical, and others, are related to some naturally occurring substances in the body—the sterols, bile acids, sex hormones, etc.

(b) The synthesis of a great number of polycyclic aromatic hydrocarbons with powerful cancer-producing ability in animals, the starting point for some of which are substances occurring naturally in the body (7, 5, 11).

(c) The demonstration that certain physiological substances (*e.g.*, the estrogens) can induce tumors in animals under specialized conditions (21). It is no longer necessary to assume that human carcinogens are all pathological compounds.

(d) The realization that cancer can be caused by a variety of agents, and is therefore not one disease but a group of diseases. In the past, the word cancer to designate a disease was more or less unconsciously employed in the sense in which the words typhoid fever or pneumonia are used instead of the broader term infectious disease. In its etiology, pathogenesis, symptomatology, pathology, and problems in diagnosis and in treatment, a carcinoma of the skin due to 3,4-benzpyrene is no more like a carcinoma of the urinary bladder due to β-naphthylamine or like a carcinoma of the stomach due to as yet unknown causes than pneumococcic pneumonia is like tuberculosis. The different cancers have in common the fundamental pathological process which is called neoplasia, just as the infectious diseases have in common the fundamental pathological processes of inflammation and immune reactions.

(e) The realization that some cancers could have more than one cause, as has been demonstrated for carcinoma of the skin and of the lung. This has its analogy in the field of infectious disease where pneumonia is caused by numerous agents.

(f) The realization that some carcinogens can cause more than one kind of cancer. The proof for this comes from experi-

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ments on lower animals at the present time. In the infectious diseases, likewise, some agents bring about more than one disease as, for example, the pneumococcus which causes pneumonia, meningitis, or peritonitis.

(g) The realization that, associated with the long-known cytological changes at a site of chronic tissue irritation, there may be chemical changes, as a result of which endogenous carcinogens may be formed.

These concepts complicate the problem of the causation of cancers, but at the same time they break it up into units each of which is approachable. It may prove possible and profitable to add, one by one, additional causes for cancers to those already known.

If such causes can be discovered and removed from the environment it may become possible to control cancer by prevention, as typhoid fever is controlled today. The immunological processes leading to recovery from an attack of typhoid fever are not fully understood, but the disease can be controlled in a population. Similarly perhaps some kinds of cancer should be controllable by prevention, even though the exact processes within the cells which give them neoplastic properties are not completely understood. Prevention of cancers should be possible if the causes are exogenous chemical agents.

In the chemically induced human tumors previously mentioned, the conditions of exposure are so extraordinary that the relationships of cause and effect can be traced even though many years elapse before the tumor appears. In contrast if one of the common visceral tumors, such as gastric carcinoma, were due to some exogenous carcinogen, it might be a substance which is so common in the environment in America and Europe as to have escaped suspicion. A careful survey of the geographic and racial distribution of tumors, or chemical analysis of many substances in the environment, might disclose valuable clues. For example the Bantu living under primitive conditions in South Africa has very little gastric cancer, according to competent observers (2); whereas in Afro-Americans, some of whom are surely Bantus, this tumor occurs. Presumably the genetic constitution has not changed entirely, and environmental factors probably determine the tumors.

Two distinct problems crystallize from these remarks:

Does chronic irritation lead to cancer because of the local presence of carcinogenic substances?

Do cancers with no obvious chemical cause have the same or different chemical causes as those which are generally recognized as being due to some chemical agent?

The next step was to examine the human body itself for the presence of carcinogenic chemicals.

In 1936 a search in man for carcinogens, exogenous or endogenous, was begun in this laboratory. Since 1938 this work has been carried out vigorously, some of it in association with Professor F. C. Koch. Many human tissues, neoplastic and nonneoplastic, as well as body secretions and excretions, have been studied or are now under investigation. Emphasis has been placed on the study of potentially cancerous tissues, and on lesions and substances found in close association with the known occurrence of human cancer. Up to the present time extracts from human livers have been most interesting, although human urine has also yielded a carcinogenic extract (33).

EXPERIMENTS WITH HUMAN LIVER

There are a number of reasons why liver was selected for analysis for the presence of carcinogens; among others are the following:

(a) The chemical relationships of the liver to the bile acids and to sterols and of these to some of the known carcinogenic hydrocarbons (10, 31).

(b) The functions of the liver in detoxification and excretion, which suggest that if chemical carcinogens are present elsewhere in the body some of them may eventually find their way to the liver.

(c) Its availability in large quantities, since it is the largest organ in the body.

(d) The frequent occurrence of cancer of the biliary system, extrahepatic and intrahepatic, which indicates that exceptional influences are acting on the cells of this tubal system. The incidence of this type of cancer in America is low in comparison with some other parts of the world (2, 3). Nevertheless, even here, a cell or group of cells in this system has a greater chance of becoming cancerous during the life of the individual than any other cells in the body, possibly excepting those of the uterine cervix. Even gastric epithelial cells, considered as individuals, give rise to cancer less commonly. The ratio of epithelial cells in the extrahepatic bile passages to those in the stomach is approximately 1:300, while the ratio of cancers in these locations is about 1:60 (36).

Experiment 1. Nonsaponifiable Lipid Extract of Human Cancer Livers

The livers from persons with cancer of various types were extracted by a method planned for recovering substances of the carcinogenic hydrocarbon group. This method involved saponification followed by extraction with ethylene dichloride. The procedure was:

Preservation of the liver in one volume of 95 per cent alcohol after grinding.
Addition of water—volume equal to that of the alcohol.
Addition of 10 gm. of KOH per 100 gm. of liver tissue.
Saponification for 18 to 24 hours on a steam bath under a reflux condenser.
Extraction with ethylene dichloride; repeated 3 times.
Dehydration with anhydrous Na_2SO_4 followed by filtration.
Evaporation to dryness at reduced pressure.
Resaponification of the extract for 4 hours with alcoholic KOH, without water. One-half gm. of KOH for each 100 gm. of liver tissue, original weight, was used.
Extraction 4 times with ethylene dichloride and evaporation of the pooled extracts to dryness.

In this experiment 9,420 gm. of liver obtained from 8 persons with carcinomas was extracted. The livers contained no gross or microscopic tumor except one that had small scattered metastases. The primary tumors were located in the stomach (3 cases), lung (2 cases), esophagus, pancreas, and rectum (1 case of each). The yield in crude extract was 65.6 gm. Additional information regarding similar extracts has been published (34).

This extract was tested in mice for cancer-producing ability by a single subcutaneous injection of 0.5 gm. dissolved or suspended in 0.5 cc. of sesame oil. These mice were of our partly inbred albino stock in

which spontaneous sarcoma, other than lymphosarcoma, has not been known to occur.[1] They were of both sexes and were 55 to 83 days old at the time of injection.

This extract proved caustic in the subcutaneous tissues and many mice developed sloughs. Others had fluctuant masses at the site of injection for many months. The first tumor at the site of injection appeared in the 6th month and the mouse died in the 7th month (182 days). Thereafter other tumors developed in rapid order as indicated in Table I. The percentage yield in the effective total tested was 32.4 per cent (12 tumors in 37 mice). All of these tumors were in males.[2]

The tumors were spindle celled or pleomorphic celled sarcomas. They grew rapidly and infiltrated the overlying skin and the underlying muscle and fascia. They appeared to arise directly in the residue of the extract which was injected. In one mouse 2 tumors appeared to have arisen independently. In another, one tumor metastasized to the lung. Attempts at serial transplantation, carried out with 3 tumors, were successful in 2. The microscopic appearance of the tumors remained unchanged during 4 transplant generations.

Table I: Carcinogenicity of Nonsaponifiable Lipid Extracts of Human Livers

		Time in months																
		0	6	7	8	9	10	11	12	13	14	15	18	21	24	27	30	33
Experiment 1	Total mice	56	42	37	34	31	21	20	15	13	10	8	6	4	4	3	1	0
	Male mice	47	33	28	25	22	12	11	6	4	1	1	1	0	0	0	0	0
	Female mice	9	9	9	9	9	9	9	9	9	9	7	5	4	4	3	1	0
	Dead with sarcoma	0	0	1	4	6	7	8	10	12	12	12	12	12	12	12	12	12
Experiment 2	Total rats, male	24	18	15	15	13	9	9	9	9	7	6	3	0	..	..	..	..
Experiment 3	Total mice	63	59	54	51	47	44	40	35	32	28	26	16	2	0	..	..	..
	Male mice	31	30	26	23	19	17	14	10	7	5	5	0	0	0	..	..	..
	Female mice	32	29	28	28	28	27	26	25	25	23	21	16	2	0	..	..	..
	Dead with sarcoma	0	0	0	0	0	0	0	1	2	3	3	4	5	5	..	..	..
Experiment 4	Total rats	26	24	24	24	21	21	21	20	18	17	15	14	10	0	..	..	..
	Male rats	12	11	11	11	10	10	10	10	9	8	6	5	3	0	..	..	..
	Female rats	14	13	13	13	11	11	11	10	9	9	9	9	7	0	..	..	..

Experiment 2. Nonsaponifiable Lipid Extract of Human Cancer Livers (Rats)

In this experiment the same specimen of liver extract as that used in experiment 1 was tested in rats for carcinogenicity. Twenty-four young male rats, from a strain inbred in this laboratory by brother to sister matings for 8 generations, were used. Each was injected subcutaneously once with 1 gm. of the liver extract in 1 cc. of sesame oil.

This amount of material caused necrosis with sloughing in some rats but not all, several showing large amounts of retained injected material at autopsy. In none did a tumor appear at the site of injection. One rat, dying in the 6th month after injection, had a mesenteric lymphosarcoma which was not considered an induced tumor. The survival time of these rats is indicated in Table I.

Experiment 3. Nonsaponifiable Lipid Extract of Human Noncancer Livers

An extract was prepared from the pooled livers of 7 persons who died without cancer but with the following main diagnoses: hypertensive heart disease (2 instances), purulent meningitis, chronic glomerulonephritis, acute hemorrhagic pancreatitis, lobar pneumonia, and bronchopneumonia. This extract was prepared by the methods used in experiment 1. From 9,045 gm. of fresh liver the yield of nonsaponifiable lipid extract was 95.0 gm.

This was injected subcutaneously into mice of both sexes, of the stock used in experiment 1 and under identical conditions, except that each mouse received 2 injections, 9 weeks apart. Each injection consisted of about 250 mgm. of the extract in 0.5 cc. of the same sesame oil. Thus while each mouse received a total of 500 mgm. of the extract as in experiment 1, it also had twice as much sesame oil, a total of 1 cc. The dose of extract was divided and diluted to reduce sloughing and extrusion. This result was apparently achieved, as large amounts of extract were seen at autopsy.

The mice lived to the ages shown in Table I. Five (4 males and 1 female) developed sarcomas at the

[1] The mice used in all the experiments reported in this paper were from this stock. A few data on their susceptibility to tumor induction by methylcholanthrene and by crude urine extracts have been reported (33).

[2] In a preliminary report of this experiment (35) 13 sarcomas were mentioned. One of these is now disqualified as possibly an undifferentiated carcinoma and not surely induced.

site of injection. In gross and microscopic appearance these tumors resembled those seen in experiment 1. The first animal died with a tumor in the 12th month. The induction time was therefore longer than that for the cancer liver extract, and the percentage yield was lower, 14.3 per cent. These indicate that the carcinogenicity of this extract was low.

Experiment 4. Nonsaponifiable Lipid Extract of Human Noncancer Livers (Rats)

Twenty-six rats were injected with the liver extract used in experiment 3. The other conditions of the experiment were the same as those of experiment 2 except that rats of both sexes were used, 12 males and 14 females. Each received 1 gm. of extract in 1 cc. of sesame oil, given once. These rats also extruded part of the extract after injection, but most of them had generous amounts at autopsy. They lived to the ages shown in Table I. None developed tumors at the site of injection.

Experiment 5. Benzene Extract of a Human Liver

A liver weighing 1,250 gm., from a white woman 62 years of age who died of carcinoma of the stomach, was extracted with benzene according to the method of Schabad (30), slightly modified as to the temperature at which the extractions were carried out. This liver contained a few scattered tumor metastases. It was put through a grinder 6 times, after which it was extracted with freshly distilled, thiophene-free benzene. Eight extractions were made at room temperature, each with 1 liter of benzene. Next an extraction was made with 6 liters of benzene, alternating at icebox and room temperatures. Finally an extraction was made with 4 liters of benzene over a period of 3 weeks. The total extract obtained by these 18 liters was 94.3 gm. of a brownish fat which was semiliquid at room temperature.

This extract was injected subcutaneously into 32 mice (15 males and 17 females) in 0.5 cc. amounts. At 4, 8, 12, and 16 weeks after the first injection the dose was repeated for each mouse and in the same location. Thus each mouse received a total of 2.5 cc. in 5 doses during a period of 16 weeks.

This extract was well tolerated, the mice rarely showing sloughs or sinuses, but usually having soft, fluctuant masses at the site of injection. The results are shown in Table II. No sarcomas occurred in the subcutaneous tissues, although 14 mice survived for 12 months, 9 for 15 months, and 4 for 18 months.

Experiment 6. Fractions of Benzene Extracts of Human Cancer Livers [3]

Although no tumors were produced at the injection site by any extract, experiments 6 A, B, C, D, E are reported because others may thus avoid unplanned duplication of effort; because they show that the tumors in experiments 1 and 3 were not induced by nonspecific irritation by tissue extracts; and because of the tumors which occurred at a distance from the injection site.

Table II: Carcinogenicity of Miscellaneous Human Tissue Extracts

	Sex of mice	Time in months: 0	3	6	9	12	15	18	21	24	27	30	Induced sarcomas
Experiment 5	Male	15	15	11	6	4	3	0	0	0	..	..	0
	Female	17	17	15	13	10	6	4	3	0	..	..	0
Experiment 6 A	Female	15	15	14	12	11	9	5	2	1	0	..	0
B	Female	15	15	15	14	13	11	8	6	4	0	..	0
C	Female	16	16	16	16	14	11	5	3	1	0	..	0
D	Male	18	18	18	16	6	3	2	2	0	..	..	0
	Female	18	17	17	16	13	12	9	5	0	..	..	0
E	Female	17	17	17	16	14	9	8	7	2	0	..	0
Experiment 7	Female	45	44	42	40	37	31	22	19	11	9	0	0
Experiment 8	Male	35	35	35	30	21	12	4	1	0	..	..	0
Experiment 9	Female	18	17	17	17	16	14	12	9	5	2	0	..

Preparation of the extracts.—12,070 gm. of liver from 11 persons with cancer was used for extraction. The cancers were primary in the following locations: stomach, 4; uterus, 2; colon, 1; ovary, 1; esophagus, 1; undetermined primary site, 1; osteogenic sarcoma, 1. Eight of these livers were free of tumor metastases on gross and microscopic examination, while 3 showed occasional small metastases not more than 1 cm. in size.

The liver tissue was dried with acetone after grinding. It was then extracted with benzene in a continuous extractor for 24 hours. This material (217 gm.)

[3] Extraction and fractionation in this experiment was performed by Professor F. C. Koch and Dr. Robert Steele of the Department of Biochemistry. I am indebted to them for permission to report this aspect of the work.

was then extracted 3 times with ether. The ether-insoluble residue (15 gm.) was used for injection in experiments 6 B, C, and D.

The ether extract (202 gm.) was extracted with saturated $NaHCO_3$ solution. The $NaHCO_3$ extract was acidified and extracted with ether. This ether extract was evaporated to dryness and yielded only 0.2 gm.

The ether extract (201.8 gm.) was treated with water, which formed an unbreakable emulsion. The ether was evaporated from the emulsion and the water suspension remaining was saturated with NaCl. The semi-solid material was filtered off and extracted with ether. The filtrate was treated with $CaCl_2$ and this solution was extracted with ether. The 2 ether extracts were combined and evaporated to dryness. The weight of the residue was 145 gm. Some of this material was used for injection in experiment 6 A.

The above-described ether-soluble extract (135 gm.) was saponified with KOH in alcohol-water solution (3:1). The alcohol was evaporated and the water solution extracted with ether. The ether extract was washed with water and evaporated to dryness. The nonsaponifiable residue (13.3 gm.) was used for injection in experiment 6 E.

Experiment 6 A. Ether-soluble fraction of a benzene extract of human cancer livers.—Each of 15 female mice, about 5 months old, was injected subcutaneously with 0.5 cc. of this extract. It was well tolerated by the tissues, and apparently almost entirely retained. The mice lived to the ages shown in Table II. There were no sarcomas in the subcutaneous tissues except 1 undifferentiated, round celled sarcoma, possibly a lymphosarcoma, infiltrating into the region of the injected extract. However, since spontaneous tumors of this kind occasionally occur in this stock, we do not consider this an induced tumor, although the possibility is not excluded.

Experiment 6 B. Ether-insoluble fraction of a benzene extract of human cancer livers.—This extract was a dark brown amorphous material, brittle when cold but plastic when warmed. It was molded into fusiform 100 mgm. pellets. These were implanted subcutaneously through a trochar into 15 female mice about 6 months old.

These pellets were caustic to the tissues and were extruded with large sloughs between the 6th and 11th days. The animals remained in good condition, and lived to the ages shown in Table II. No residual extract was seen at autopsy in any mouse. In view of these events this extract appears to have been inadequately tested for its cancer-producing ability. None of the mice developed a tumor at the point of injection.

Experiment 6 C. Ether-insoluble fraction of a benzene extract of human cancer liver.—The same extract as that used in experiment 6 B was molded into pellets of only 25 mgm. These were implanted subcutaneously through a trochar into 16 female mice about 5 months of age.

These pellets also were not tolerated by the tissues and were sloughed out between the 8th and the 25th days. The mice lived to the ages indicated in Table II. Autopsies revealed no chemical in any mouse. No tumors developed at the site of injection. This extract also was inadequately tested.

Experiment 6 D. Ether-insoluble fraction of a benzene extract of human cancer livers.—The same extract was used as in experiments 6 B and 6 C. Here, however, it was pulverized at icebox temperature and implanted under the skin through a trochar. Each mouse received about 45 mgm., distributed along the trochar tract which ran most of the dorsal length of the mouse. Thirty-six mice, equally divided as to sex and 5 to 11 weeks of age, were so injected.

This extract also was poorly tolerated and most of the mice developed linear sloughs between the 7th and the 30th days. No tumors occurred in the subcutaneous tissues. At autopsy only a few mice showed any residual extract and these had but traces. Therefore this extract cannot be considered to have been fully tested.

Experiment 6 E. Nonsaponifiable fraction of a benzene extract of human cancer liver.—Each of 17 female mice, about 6 months of age, was injected subcutaneously with about 100 mgm. of this extract dissolved in 0.5 cc. of sesame oil. This extract appeared to be well tolerated, and to be retained without loss. The mice lived to the ages indicated in Table II. None had sarcomas at the site of injection, although many showed large amounts of injected extract.

EXPERIMENTS WITH HUMAN CANCERS

Experiment 7. Extract of Human Cancers

Fresh human tissue, weighing 7,970 gm., approximately 4,770 gm. of which was carcinoma tissue and the remainder either lung or liver, was saponified and extracted by the methods previously described. The cancer tissue was composed of the following types: approximately 400 gm. of carcinoma of the rectum metastatic in the liver; about 870 gm. of carcinoma of the pancreas; about 500 gm. of carcinoma of the bile ducts metastatic in the liver; about 3,000 gm. of primary lung carcinoma and its metastases in the mediastinum and the opposite lung.

From this tissue the nonsaponifiable lipid extract was 33.1 gm.

Each of 45 female mice, about 3 months of age, was given 1 subcutaneous injection of about 0.5 gm. of this extract in 0.5 cc. of sesame oil.

The animals lived to the ages shown in Table II. The extract appeared to be almost entirely retained. Nevertheless no sarcomas arose at the site of injection.

Experiment 8. Extract of Human Cancers

In this experiment the human tissues used for extraction had previously been treated by the Kaiserling method. The methods were essentially those outlined by Mallory (23) except that Kaiserling II consisted of 95 per cent alcohol and thymol was omitted from Kaiserling III.

18,435 gm. of tissue composed of the following was used:

Retroperitoneal spindle cell sarcoma	1,585 gm.
Lymphosarcoma of the abdomen	2,940 "
Primary leiomyosarcoma of the pleura	1,150 "
Leukosarcoma of the mesentery	1,100 "
Primary peritoneal mesothelioma	1,110 "
Peritoneal mesothelioma metastatic to the liver (estimated 66 per cent tumor)	3,650 "
Cancer of the breast metastatic to liver (estimated 90 per cent tumor)	5,000 "
Cancer of the pancreas metastatic to liver (estimated 80 per cent tumor)	850 "
Malignant melanoma metastatic to the liver (estimated 95 per cent tumor)	650 "
Primary carcinoma of the stomach, plus Primary carcinoma of the jejunum, plus Adenomatous hyperplasia of the prostate, total of	400 "

This pooled tissue was saponified and extracted by the method previously described. The yield of nonsaponifiable lipids was 93.6 gm. From this, 56.4 gm. of cholesterol was removed by crystallization and recrystallization from boiling 95 per cent ethyl alcohol. After evaporation a residue of 37.2 gm. of extract remained which was used for injection.

Thirty-five male mice from 4 to 6 weeks of age were injected subcutaneously with this extract. An initial injection of about 250 mgm. in 0.5 cc. of sesame oil was followed by sloughs through which apparently most of the extract was extruded. Consequently 3 additional injections were given, 8, 12, and 16 weeks after the first dose. Each consisted of approximately 100 mgm. of extract in 0.1 cc. of sesame oil. They were moderately well tolerated. At autopsy nearly every mouse showed residual injected extract, varying in amount from traces to large masses.

The mice lived to the ages indicated in Table II. None had sarcomas at the site of injection.

Experiment 9. Sesame Oil

In all experiments reported in this paper in which sesame oil was used as a vehicle or solvent, the specimen of sesame oil was the same. In experiments 6 E, 7, and 8, in all of which sesame oil was used, no tumors occurred at the site of injection. Unless it be assumed that the tissue extracts exerted an inhibitory action on possible tumor induction by the sesame oil, these experiments show that this sample of sesame oil was not carcinogenic. However, the oil was tested alone in this experiment.

Eighteen female mice about 8 weeks of age were injected subcutaneously with 0.5 cc. of sesame oil. One year later, no sarcomas having appeared, each mouse then living was reinjected with 0.5 cc. of sesame oil, followed 4 weeks later by another similar injection.

No tumors were induced by these injections. The mice lived to the ages shown in Table II.

Miscellaneous Tumors

In these experiments numerous tumors were encountered which arose at a distance from the site of the injected extracts. The problem is to decide how many if any of them should be counted as induced. If only tumors occurring directly at the point of application of a substance being tested for carcinogenicity are considered induced, true tumor-producing ability may be overlooked, because some substances fail to produce tumors at the site of first contact with the tissues, for example, *o*-aminoazotoluene (40) and dimethylaminoazobenzene (18). Others may induce tumors both at the point of application and at a distance, for example, 1,2,5,6-dibenzanthracene (1). It is necessary, therefore, to attempt evaluation of all tumors obtained in an experiment.

Another problem in interpretation arises in connection with tumors of the mammary gland when substances are tested for carcinogenicity by subcutaneous injection. When the injection is bulky some migration, with possible direct contact with mammary gland epithelium, is inevitable regardless of the site selected. Shall mammary gland tumors be considered induced if the chemical is demonstrated to be in relation to them? Strong and Smith (37) and Bonser and Orr (4) have reported induction of mammary gland tumors by direct contact with a carcinogen.

All the tumors of experiments 1 to 9 have been tabulated as to type and time of occurrence in Tables III and IV. By referring to the corresponding experiments in Tables I and II the number of animals alive at any time can be found. In some instances the number of tumors is greater than the number of mice that died because some mice had tumors of more than one type. Multiple tumors of a single type in a mouse are tabulated as one. All of the tumors were diagnosed histologically. Ovarian cysts have been omitted.

Lung tumors.—These were the common white nodules, which microscopically were usually papillary adenomas or carcinomas, although some had a sar-

TABLE III: SUMMARY OF ALL TUMORS IN EXPERIMENTS 1 AND 3

	Sex	Type of tumor	Time in months: 6	7	8	9	10	11	12	13	14	15	18	21	24	27	30	33	Total tumors
Experiment 1	Male	Lung							1					1					2
		Lymphatic	1																1
		Subcutaneous sarcoma		1	3	2	1	1	2	2									12
	Female	Lung												1		1		1	3
		Lymphatic										1	1						2
		Mammary gland												1		1	2		4
		Uterus															1		1
		Ovary																1	1
Experiment 3	Male	Lung			1		1		2	1			1						6
		Lymphatic		1															1
		Subcutaneous sarcoma							1	1	1		1						4
	Female	Lung													1				1
		Lymphatic	1	1															2
		Subcutaneous sarcoma												1					1
		Mammary gland	1					1	1		2	1	1						7

TABLE IV: SUMMARY OF ALL TUMORS IN EXPERIMENTS 5, 6, 7, 8, AND 9

	Sex	Type of tumor	Time in months: 3	6	9	12	15	18	21	24	27	30	Total tumors
Experiment 5	Male	Lung				1		2					3
		Lymphatic			1			1					2
	Female	Lung								1			1
		Lymphatic		1	1	1	1						4
		Mammary gland			1	1	1	1		1			5
		Hepatic								1			1
Experiment 6 A	Female	Lung			1		1	3	1				6
		Lymphatic			1		1		1	1			4
Experiment 6 B	Female	Lung						2			3		5
		Lymphatic								1	1		2
		Mammary gland					2	1	1		2		6
		Liver							1				1
Experiment 6 C	Female	Lung					1	1		1			3
		Lymphatic				2		2		1	1		6
		Mammary gland					2	3					5
Experiment 6 D	Male	Lung				1							1
		Lymphatic			1								1
	Female	Lung				1			3	1			5
		Lymphatic			1	2	1	1	1				6
Experiment 6 E	Female	Lung				1	1	1	1	2	1		7
		Lymphatic				1			1	2			4
		Mammary gland					1						1
Experiment 7	Female	Lung				1	2	4		3	1	5	16
		Lymphatic			1		3	3			1	2	10
		Mammary gland		2	1		2				1		6
Experiment 8	Male	Lung				2	1	2	1				6
		Lymphatic						1					1
Experiment 9	Female	Lung					1	2	2	1			6
		Lymphatic				1							1
		Mammary gland				1		1	1				3

coma-like stroma. A few had metastasized into the mediastinal lymph nodes, and some showed invasion of bronchi, but usually they were localized parenchymal tumors.

Lymphatic tumors.—While these were not all derived from lymphocytes, they belong to the general group of tumorous diseases of the hematopoietic and lymphatic systems. Leukemias, pseudoleukemias, and lymphosarcomas of many types are included.

Mammary gland tumors.—Here are included the carcinomas and adenomas of the mammary gland. They were usually adenocarcinomas but solid carcinomas were also seen. Acantho-adenocarcinomas found in the subcutaneous tissues are included here, although their origin from mammary gland epithelium is not proved.

Uterus.—One adenocarcinoma of the uterus with metastases was seen.

Ovary.—The one tumor encountered was an adenoma.

Liver.—One adenoma of hepatic-cell type was seen.

Table V is a summary of the tumors in experiments 1 and 3 (except the induced sarcomas). Table VI summarizes the other experiments in which human tissue extracts were injected. From these two tables a comparison can be made between the miscellaneous tumors, including the mammary gland tumors, occurring after injection of tissue extracts which were locally carcinogenic and those which were not. The data in these tables were taken from Tables I to IV and only mice living more than 6 months after injection were included. Without regard to longevity of the mice the percentages of each sex that developed tumors lie within the same range. Certainly the extracts that induced sarcomas at the site of injection were associated with no more tumors at distant sites than those extracts which did not induce sarcomas.

DISCUSSION

In experiment 1 the induction time and the percentage yield of tumors indicate that the 0.5 gm. of cancer liver extract which was injected, expressed in terms of the potency of methylcholanthrene, probably contained a fraction of a milligram of active carcinogen. In experiment 3 the induction time was longer and the percentage yield lower, indicating that the potency of this noncancer liver extract was less.

Experiment 6 E, in which the nonsaponifiable lipids from the ether-soluble fraction of a benzene extract of cancer liver were used, may have given negative results because of low dosage or because of absence of a carcinogenic factor. The ether-soluble fraction of benzene extract, tested in experiment 6 A, was not carcinogenic under the conditions which prevailed, possibly for the same reasons. The ether-insoluble fraction of this same benzene extract, reported in experiments 6 B, C, and D, was negative

TABLE V: SUMMARY OF MISCELLANEOUS TUMORS

	Male mice		Female mice		Total mice	
	Number of mice	Number with tumor	Number of mice	Number with tumor	Number of mice	Number with tumor
Experiment 1	21	3	9	11	30	14
Experiment 3	26	7	28	10	54	17
Totals	47	10	37	21	84	31
Per cent		21.3		56.8		36.9

TABLE VI: SUMMARY OF MISCELLANEOUS TUMORS

	Male Mice		Female mice		Total mice	
	Number of mice	Number with tumor	Number of mice	Number with tumor	Number of mice	Number with tumor
Experiment 5	11	5	15	11	26	16
Experiment 6 A	..	..	14	10	14	10
Experiment 6 B	..	..	15	14	15	14
Experiment 6 C	..	..	16	14	16	14
Experiment 6 D	18	2	17	11	35	13
Experiment 6 E	..	..	17	12	17	12
Experiment 7	..	..	42	32	42	32
Experiment 8	35	7	..	..	35	7
Totals	64	14	136	104	200	118
Per cent		21.9		76.5		59.0

also. This fraction was inadequately tested since it was not retained by the animals. The experiments are reported, however, because of their information on the incidence of miscellaneous tumors in these mice.

The failure to detect carcinogenic substances in human liver by the methods of Schabad (experiment 5) is probably not significant since only a single experiment was made, and unpublished data indicate that the carcinogen is absent in many livers.

Carcinogenic activity by liver extracts has now been reported by 4 groups, working in Russia, South Africa and London, France, and Chicago (35). Schabad and his co-workers, in a series of publications from Russia since 1937, report tumor production by benzene extracts of liver. Their summary (19) shows 3 sarcomas and 1 carcinosarcoma at the site of injection in 162 mice surviving for 8 months. In 106 mice injected with benzene extracts from noncancer livers and living over 8 months no sarcomas occurred at the site of injection. More recently (20) this group has reported results of experiments in which the nonsaponifiable lipid fraction of benzene extracts was injected. One sarcoma was produced at the site of injection in 13 mice living for 8 months.

Des Ligneris, working in South Africa, has tested nonsaponifiable lipid extracts from human livers (8). The livers were taken from noncancerous Europeans living in South Africa, and from Bantus both cancerous and noncancerous. Both of the latter types of extract induced tumors when applied to the skins of mice. In 237 mice surviving for 8 months, 7 malignant tumors were found.

Des Ligneris sent some of his extracts to London where they were tested by Hieger (14) who also prepared other tissue extracts. Following subcutaneous injections into 367 mice 12 sarcomas have developed. Curiously, in contrast with the results reported here, 11 of the 12 tumors were in female mice.

Sannié, Truhaut, and Guérin (29), using the nonsaponifiable lipid extract of human cancer livers, induced 6 sarcomas at the site of injection in 20 mice that lived 1 year or more. A similar extract from the livers of cancer-free persons produced no tumors at the site of injection.

All of these groups have now reported the carcinogenic activity to be present in the nonsaponifiable lipid extract, although Schabad and his co-workers originally used benzene extracts.

There is disagreement whether livers from noncancerous persons contain the carcinogen. Thus in Kleinenberg's reports and in the report by Sannié and his associates no sarcomas were induced at the site of injection of noncancer extracts, although both groups of workers reported that the incidence of tumors at distant sites was increased over controls. Des Ligneris and Hieger reported carcinogenic activity in liver extracts from noncancerous Bantus, but the former stated that similar extracts from noncancerous Europeans living in South Africa gave negative results. In experiment 3, reported here, there was carcinogenic activity in the liver extract from noncancerous Americans. These differences may be real, and dependent on dietary or geographic factors, or they may disappear on further investigation. The question is being further studied with a larger number of human noncancer livers, each individually extracted and injected. A preliminary comparison of the yield of the crude extract from cancer and noncancer livers offers no aid in solving this problem (34).

Considerable emphasis has been placed on an analysis of the tumors occurring at a distance from the site of injection and of the mammary gland tumors, because two of the groups working with liver extracts report a greater number of such tumors in mice injected with cancer liver extracts than in those injected with noncancer liver extracts and in controls (19, 20, 29). The results reported in this paper are not in accordance with their findings.

The failure to detect carcinogenic substances in cancers (experiments 7 and 8) was disappointing because the extracts were prepared from large amounts of tissue. The report of Menke (25) is of interest in this connection. He extracted small mammary gland cancers with lipid solvents, and with the extracts induced spindle-cell sarcomas at the point of injection in mice. In retrospect, the use of large amounts of cancer tissue and especially the use of metastases for extraction in seeking carcinogenic substances, might have been a disadvantage, because the bulky growth might have served to dilute any carcinogen originally present. Also, according to the view that cancers are autonomous after they are started and are independent of the continued action of the inducing agent, metastatic lesions need not necessarily contain any carcinogen.

The chemical nature of the carcinogen found in the liver is unknown, other than that it belongs in the general group of the nonsaponifiable lipids. Its origin, whether exogenous or endogenous, likewise has not been determined. An exogenous origin is not excluded because of its presence in the liver. These points are now under investigation.

SUMMARY

Twelve sarcomas were induced in 37 mice at the site of subcutaneous injection of the nonsaponifiable lipid fraction extracted from pooled noncancerous livers of persons who died with cancer. The induction time was 182 days and the percentage yield was 32.4 per cent.

An extract similarly prepared from the livers of noncancer-bearing persons had less carcinogenic activity, having an induction time of 12 months and a percentage yield of 14.3 per cent. Five sarcomas were induced in 35 mice. Neither of these 2 extracts induced tumors in rats.

A benzene extract of cancer liver, and various fractions obtained from a benzene extract of cancer livers failed to induce tumors at the site of injection.

Extracts of cancer tissues also did not induce tumors.

A theory for the chemical causation of cancer is outlined, and its possible relationship to the theory of chronic irritation is pointed out.

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REFERENCES

1. Andervont, H. B. Pulmonary Tumors in Mice. I. Susceptibility of the Lungs of Albino Mice to the Carcinogenic Action of 1:2:5:6-Dibenzanthracene. Pub. Health Rep., **52**:212-221. 1937.
2. Berman, C. Malignant Disease in the Bantu of Johannesburg and the Witwatersrand Gold Mines. South African J. M. Sc., **1**:12-30. 1935.
3. Bonne, C. Cancer and Human Races. Am. J. Cancer, **30**: 435-454. 1937.
4. Bonser, G. M., and Orr, J. W. The Morphology of 160 Tumours Induced by Carcinogenic Hydrocarbons in the Subcutaneous Tissues of Mice. J. Path. & Bact., **49**:171-183. 1939.
5. Cook, J. W., and Haslewood, G. A. D. The Synthesis of 5:6-Dimethyl-1:2:-benzanthraquinone, a Degradation Product of Deoxycholic Acid. J. Chem. Soc., 428-433. 1934.
6. Cook, J. W., Hewett, C. L., and Hieger, I. The Isolation of a Cancer-Producing Hydrocarbon from Coal Tar. J. Chem. Soc., 395-405. 1933.
7. Cook, J. W., and Kennaway, E. L. Chemical Compounds as Carcinogenic Agents. Second Supplementary Report: Literature of 1938 and 1939. Am. J. Cancer, **39**:381-428, 521-582. 1940.
8. Des Ligneris, M. J. A. The Production of Benign and Malignant Skin Tumours in Mice Painted with Bantu Liver Extracts. Am. J. Cancer, **39**:489-495. 1940.
9. Dreyfus, J. R. Lungencarcinom bei Geschwistern nach Inhalation von eisenoxydhaltigem Staub in der Jugend. Ztschr. f. klin. Med., **130**:256-260. 1936.
10. Fieser, L. F. Cause and Growth of Cancer. University of Pennsylvania Bicentennial Conference. Philadelphia: University of Pennsylvania Press. 1941.
11. Fieser, L. F., and Newman, M. S. Methylcholanthrene from Cholic Acid. J. Am. Chem. Soc., **57**:961. 1935.
12. Haagensen, C. D. An Exhibit of Important Books, Papers, and Memorabilia Illustrating the Evolution of the Knowledge of Cancer. Am. J. Cancer, **18**:42-126. 1933.
13. Härtung, F. H., and Hesse, W. Der Lungenkrebs, die Bergkrankheit in den Schneeberger Gruben. Vierteljahrsschrift für gerichtliche Medicin und öffentliches Sanitätswesen, **30**:296; **31**:102. 1879.
14. Hieger, I. The Examination of Human Tissue for Carcinogenic Factors. Am. J. Cancer, **39**:496-503. 1940.
15. Holleb, H. B., and Angrist, A. Bronchiogenic Carcinoma in Association with Pulmonary Asbestosis. Am. J. Path., **18**:123-131. 1942.
16. Hueper, W. C., Briggs, F. A., and Wolfe, H. D. Experimental Investigation on the Etiology of Aniline Tumors. J. Indust. Hyg. & Toxicol., **20**:85-91. 1938.
17. Hutchinson, J. On Some Examples of Arsenic Keratoses of the Skin and Arsenic Cancer. Tr. Path. Soc. London, **39**:352-363. 1887-88.
18. Kinosita, R. Studies on the Cancerogenic Chemical Substances. Tr. Soc. path. jap., **27**:665-727. 1937.
19. Kleinenberg, H. E., Neufach, S. A., and Schabad, L. M. Endogenic Blastogenic Substances. Am. J. Cancer, **39**: 463-488. 1940.
20. Kleinenberg, H. E., Neufach, S. A., and Schabad, L. M. Further Study of Blastomogenic Substances in the Human Body. Cancer Research, **1**:853-859. 1941.
21. Lacassagne, A. Apparition de cancers de la mamelle chez la souris mâle, soumise à des injections de folliculine. Compt. rend. Acad. d. sc., **195**:630-632. 1932.
22. Lynch, K. M., and Smith, W. A. Pulmonary Asbestosis. III. Carcinoma of Lung in Asbesto-Silicosis. Am. J. Cancer, **24**:56-64. 1935.
23. Mallory, F. B. Pathological Technique. Pp. 380-381. Philadelphia: W. B. Saunders Company. 1938.
24. Martland, H. S. The Occurrence of Malignancy in Radioactive Persons. Am. J. Cancer, **15**:2435-2516. 1931.
25. Menke, J. F. A Preliminary Note on the Extraction of a Carcinogenic Factor from Primary Human Mammary Cancer. Science, **92**:290-291. 1940.
26. Paris, J. A. Pharmacologia; or the History of Medicinal Substances, with a View to Establish the Art of Prescribing. New York: F. and R. Lockwood. 1822. Quoted from Haagensen (12).
27. Pirchan, A., and Šikl, H. Cancer of the Lung in the Miners of Jáchymov (Joachimstal). Am. J. Cancer, **16**: 681-722. 1932.
28. Rehn, L. Blasengeschwülste bei Fuchsin-Arbeitern. Arch. f. klin. Chir., **50**:588-600. 1895.
29. Sannié, Ch., Truhart, R., and Guérin, P. Production de sarcomes chez la souris par injections d'un extrait obtenu a partis de foies de malades cancereux. Bull. Assoc. franc. p. l'étude du cancer, **29**:106-121. 1941.
30. Schabad, L. M. Production expérimentale de tumeurs malignes par un extrait benzénique du foie d'un cancéreux. A propos de la question des substances cancérigènes endogènes. Compt. rend. Soc. de biol., **124**:213-216. 1937.
31. Shear, M. J., and Leiter, J. Studies in Carcinogenesis. XV. Compounds Related to 20-Methylcholanthrene. J. Nat. Cancer Inst., **2**:99-113. 1941.
32. Southam, A. H., and Wilson, S. R. Cancer of the Scrotum. Brit. M. J., **2**:971-973. 1922.
33. Steele, R., Koch, F. C., and Steiner, P. E. The Extraction of a Carcinogenic Fraction from Human Urine. Cancer Research, **1**:614-617. 1941.
34. Steiner, P. E. The Nonsaponifiable Lipid Fraction of Livers from Cancerous and Non-cancerous Persons. Am. J. Path., **17**:667-671. 1941.
35. Steiner, P. E. A Cancerogenic Tissue Extract from Human Sources. Science, **92**:431-432. 1940.
36. Steiner, P. E. Unpublished data.

37. STRONG, L. C., and SMITH, G. M. The Local Induction of Carcinoma of the Mammary Gland with Methylcholanthrene. Yale J. Biol. & Med., **11**:589-592. 1939.

38. TELEKY, L. Der berufliche Lungenkrebs. Acta, Union internat. contre cancer, **3**:253-275. 1938.

39. VON VOLKMANN, R. Beiträge zur Chirurgie, anschliessend an einen Bericht über die Thätigkeit der chirurgischen Universitäts-Klinik zu Halle im Jahre 1873. Leipzig: Breitkoff und Härtel. 1875. Quoted from Haagensen (12).

40. YOSHIDA, T. Studien über die Entwicklung des experimentellen Hepatoms durch o-Amidoazotoluol, besonders die stufenweise Verfolgung der Leberveränderung bis zur Carcinomentstehung. Tr. Soc. path. japon., **24**:523-530. 1934. See summary in English by SHEAR, M. J. Studies in Carcinogenesis. IV. Development of Liver Tumors in Pure Strain Mice Following the Injection of 2-Amino-5-Azotoluene. Am. J. Cancer, **29**:269-284. 1937.

Studies on Carcinogenesis in Rabbits

I. Malignant Tumors Induced in Cottontail Rabbits by the Injection of Methylcholanthrene in Tricaprylin*

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New impetus to the investigation of experimental cancer has been given by studies of the papilloma-to-carcinoma sequence that is frequently observed in domestic (13, 12) and cottontail rabbits (20, 6, 9, 19) following the production of papillomas with Shope's virus (18). While making it clear that the nature of the relationship of the virus to the cancer is not understood, these studies have yielded evidence that the rabbit has many advantages over the rat and the mouse as a host in which to investigate cancer. Accordingly, we have attempted to induce nonvirus tumors in rabbits by using chemical compounds of known purity in order to compare them with virus tumors. That methylcholanthrene is a most effective carcinogenic agent in rabbits has been briefly recorded (21). The work on which this preliminary report [1] was based has been amplified by further experimentation and will be described in a series of papers.

The present paper is limited to a description of the production of sarcomas and carcinomas in cottontail rabbits by the use of methylcholanthrene. These rabbits received methylcholanthrene only; *i.e.*, none of our animals receiving both carcinogenic agent and virus are included in the present report. The methylcholanthrene was incorporated in the triglyceride, tricaprylin, and brought into prolonged contact with the rabbits' tissues.

The rabbit has been but little used for studying carcinogenic agents because of the low incidence and the long latent period that have characterized the production of tumors in this host. This concept of the rabbit's limited reactivity, or refractoriness, to carcinogenic agents has grown out of the use of tar, 1,2,5,6-dibenzanthracene, and 3,4-benzpyrene. The literature that pertains to the carcinogenic effects of tar on a variety of hosts including the rabbit has been reviewed by Woglom (22) and by Seelig and Cooper (17). That certain tars will induce cancers in rabbits is well known, but cancers result only after the repeated application of the tar to the skin over prolonged periods of time. Cook and Kennaway (4) and Fieser (5), in more recent surveys of the literature on carcinogenic hydrocarbons, give it as their view that mice and rats are the animals of choice for testing the carcinogenic potentialities of such agents.

Except for such tumors as have resulted from tarring the ears alone, few tumors have been induced in rabbits by carcinogenic agents. Thus, Burrows and Boyland (3), who summarized the literature through 1937, found 198 treated rabbits of which only 4 developed cancers. These 4 rabbits were in a group of 112 that had received 1,2,5,6-dibenzanthracene. The tumors developed after prolonged periods of time, ranging from 308 to 2,198 days. No tumors appeared in either the group of 47 rabbits that received 3,4-benzpyrene, or the group of 39 that received tar alone or tar and a sex hormone. Klinke (10), however, observed that mixed-cell sarcomas developed in 4 of 42 rabbits in from 224 to 448 days after repeated intramuscular injections of benzpyrene in fat, and Schürch (16) reported 11 cancers in 20 animals that appeared in from 390 to 547 days (average 27 months) after the ears were painted with 3,4-benzpyrene thrice a week. Although methylcholanthrene is recognized as one of the most potent of the carcinogenic agents, its effects on rabbits have not been described.

The production of cancers in rabbits following the use of both a carcinogenic chemical and a tumor-producing virus has been reported. In such experiments it is obviously difficult to evaluate the exact part played by each agent. Rous and Kidd (14) have concluded from their experiments that the intravenous injection of papilloma virus accelerates the development of malignancy in tar-induced cutaneous tumors. Lacassagne and Nyka (11) have attributed the malignant changes observed in benzpyrene-induced tumors, initiated over 500 days previously, to Shope papilloma virus injected intravenously 44 days earlier (a highly improbable situation). Finally, Andrewes *et al.* (1, 2) have reported finding single sarcomas in each of 2 rabbits after repeated intramuscular injections of tar and a single intravenous injection of Shope fibroma virus. These sarcomas, they felt, were nonvirus in nature.

MATERIALS, METHODS, AND RESULTS

Experimental Procedure

Western cottontail rabbits (*Sylvilagus floridanus alacer* Bangs) from southern Kansas were used as recipients for the carcinogenic agent, methylcholanthrene (m.p. 180–181° C.).[2] Each of 11 rabbits was given 1 gm. of methylcholanthrene. The carcinogenic agent was injected into each of 4 sites, subcutaneously into

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[1] Presented at Pittsburgh, Pa., before the meeting of the American Association for Cancer Research, March 20, 1940.

[2] Procured from the Chemical Sales Division, Eastman Kodak Company, Rochester, N. Y.

both axillary regions, and intramuscularly into the hamstring muscles of both thighs, in an amount per site of 250 mg. in 1 ml. of tricaprylin (m.p. 4–6° C.).[3, 4] Following injection, each animal was examined at weekly intervals for the duration of its natural life. The diagnosis was established in each instance by necropsy and histopathological examination. Paraffin sections were routinely stained with hematoxylin and eosin.

For transplantation, only such portions of the tumor as showed no evidence of degenerative changes were selected and finely cut in Locke's solution to yield blocks of tissue small enough to pass through the lumen of a hypodermic needle, gauge 12. From 10 to 20 fragments in 0.5 ml. of Locke's solution were injected into each of 6 sites, 2 subcutaneous (both axillary regions) and 4 intramuscular (upper extensor muscles of each limb). Both local and western cotton-tail rabbits were used as recipients for the tissue transplants. These animals were examined every 2 weeks for 2 months and thereafter every month for an additional 6 months.

Results

Since 5 of the 11 host animals died from intercurrent infection or injury before the 175th day, at which time no histological evidence of neoplasia was found, they will not be further considered. Each of the remaining 6 animals had one or more malignant tumors at the time of death, which occurred in from 176 to 295 days after injection. Of these 6 rabbits, 4 had soft-tissue sarcomas with metastases in 2, 1 an epidermoid carcinoma, and 1 both an epidermoid carcinoma and 4 soft-tissue sarcomas. These rabbits are described below in the order of their survival. The results are summarized in Table I. To facilitate recording, the sites of injection and the tumors that resulted are designated in the protocols as follows:

Right axillary region (subcutaneous) = site I, tumor A.

Hamstring muscles of right thigh (intramuscular) = site II, tumor B.

Left axillary region (subcutaneous) = site III, tumor C.

Hamstring muscles of left thigh (intramuscular) = site IV, tumor D.

Rabbit No. 1.—Rabbit No. 1 was injected on June 13, 1939. On the 147th day after injection, a soft swelling (tumor A) measuring 0.5 × 1 × 1 cm. was discovered at site I. When the animal died a month later, on the 176th day, the tumor had enlarged, measuring 2 × 3.5 × 2 cm., and softened. Although it was firmly attached to the overlying skin, the epidermal surface was intact. At necropsy a purulent necrotizing pneumonia of both lower lobes, generalized peritonitis, and hepatic cirrhosis were revealed. *Pasteurella cuniculicida* was recovered from the blood, the lung, and the peritoneal exudate. No metastases were found, nor was there any evidence of tumor formation at the other sites of injection. Microscopic examination of tumor A showed it to be an epidermoid carcinoma (Fig. 1) which had infiltrated the pectoral and intercostal muscles.

Rabbit No. 2.—Rabbit No. 2 was injected on June 13, 1939. On the 35th day after injection, a diffuse invasive growth (tumor A) was found at site I. It had infiltrated the surrounding musculature to immobilize the shoulder joint partially, thereby markedly altering the animal's stance. One month later, on the 63rd day, tumor A had enlarged to 1.5 × 3 × 1 cm. in its superficial discrete portion; the deeper infiltrative portion could not be measured. Firm nodules approximating 0.5 × 1.2 × 0.5 cm. in size were discovered at the other 3 sites of injection. When the animal died 3 months later, on the 226th day after injection, tumor A measured 2.5 × 4.5 × 1.5 cm. It was soft and superficially hemorrhagic. Its general consistency and appearance was that commonly associated with sarcomatous

Table I: Production of Sarcomas and Carcinomas in Rabbits by Methylcholanthrene in Tricaprylin

(Rabbits Arranged in Order of Survival)

Rabbit number	Number of days between injection and necropsy	Histopathological diagnosis	Extent of involvement: Primary tumors, Injection site * I	II	III	IV	Metastases: Nodes	Lungs	Kidneys	Liver
1	176	Carcinoma	+	0	0	0	0	0	0	0
2	226	Sarcoma	+	+	+	+	+	0	0	0
3	228	Sarcoma	0	+	0	0	0	0	0	0
4	244	Sarcoma	+	+	+	+	+	+	+	+
5	255	Sarcoma	+	+	+	+	0	0	0	0
6	295	Sarcoma	+	+	+†	+	0	0	0	0
		Carcinoma	..	..	+	..	..	..	..	..

* Site I, right axillary region (subcutaneous).
Site II, hamstring muscles of right thigh (intramuscular).
Site III, left axillary region (subcutaneous).
Site IV, hamstring muscles of left thigh (intramuscular).
† Both an epidermoid carcinoma and a soft-tissue sarcoma developed at site III.

[3] We used this synthetic triglyceride, tricaprylin, because of Fieser's suggestion (5) that it is highly desirable to administer carcinogenic hydrocarbons in a solvent of known chemical characteristics.

[4] Other experiments currently in progress in our laboratory are showing that methylcholanthrene in tricaprylin is equally effective in producing malignant tumors in the common domestic rabbit (genus *Oryctolagus*).

degeneration. Tumors B, C, and D were firm and approximated 0.6 × 1 × 0.6 cm. in size. At necropsy there was found a bilateral purulent pyelitis, abscesses in the cortical areas of both kidneys, hepatic cirrhosis, and the replacement of the right axillary lymph nodes by a well-encapsulated, cream-colored, purulent exudate. *Pasteurella cuniculicida* was recovered from the heart's blood, and from the abscessed kidneys and lymph nodes.

A histologic examination was made of the primary tumors, the lymph nodes, the lungs, the kidneys, the spleen, and the bone marrow. Tumor A was a spindle-cell sarcoma of the large-cell variety in which there were two small islets of the mixed-cell type. Approximately one-sixth of the tumor was necrotic. The tumor had infiltrated between the ribs to reach the pleura. Tumor B was a mixed-cell sarcoma with about two-thirds of its central portion necrotized. It had involved the surrounding musculature through replacement, the inclusion of strands of muscle, and hyaline degenerative changes. Both tumor C and tumor D were soft-tissue sarcomas in which were incorporated large spindle-cell and mixed-cell varieties of sarcomatous tissue. Tumor D had extensively invaded and replaced the musculature (Fig. 2). The only metastasis discovered was in the left inguinal node, which measured 0.8 × 1.2 × 1 cm. This node had been almost entirely replaced by highly vascularized sarcomatous tissue consisting of both the large spindle-cell and polymorphic varieties (Fig. 3).

Rabbit No. 3.—Rabbit No. 3 was injected on May 16, 1939. On the 28th day after injection, all 4 sites of inoculation were indurated. This induration disappeared, however, and no changes were noted during the next 6 months. The animal died 228 days after injection. At autopsy a tumor (tumor B) was found (site II) embedded in the musculature which it had infiltrated and replaced. Its size was approximated at 0.5 × 0.5 × 1 cm. The 3 other sites of inoculation showed slight discoloration but no evidence of tumor formation. No enlarged lymph nodes or evidence to suggest metastatic foci was discovered. Death was attributed to a pneumonia, *Pasteurella cuniculicida* being recovered from the pulmonary tissue. On microscopic examination tumor B was found to be a mixed-cell sarcoma within which there were several islets of tissue containing methylcholanthrene crystals. Specimens of tissue from the other sites of injection were not saved for histologic study.

Rabbit No. 4.—Rabbit No. 4 was injected on June 13, 1939. On the 77th day after injection, a soft, movable swelling (tumor C) measuring 1.5 × 2.5 × 2 cm. was present in the subcutaneous tissue at site III. Its consistency was like that of an abscessed, necrotic lymph node. Although the overlying skin was discolored from the extravasation of blood, it did not appear to be attached to the tumor. During the next 3 months, tumor C enlarged slightly and became softer. Seven months after injection changes were also apparent at 2 more of the 4 sites of injection: tumor A was a subcutaneous, argillaceous nodule measuring 0.8 × 1 × 1 cm.; tumor B was an irregular nodular mass approximating 2.5 × 3 × 2.5 cm.; and tumor C had increased in size to 2 × 3 × 2.5 cm. The animal died a month later (244 days after injection), following several weeks of progressive loss of weight and cachexia. At necropsy a greenish "pellet" of methylcholanthrene in tricaprylin was seen lying superficially in the subcutaneous tissue at site I (Fig. 4). Directly underneath this pellet a firm nodule (tumor A) measuring 1 × 1.8 × 0.4 cm. was found. Tumor B (Fig. 5a) had grown to 3.5 × 9.2 × 4.5 cm. and had infiltrated the muscle to surround the bones. On section (Fig. 5b) this tumor showed areas of irregular density with circular, softened areas distributed through firm fibrous tissue. Tumor C (Fig. 6) was a discrete, soft, hemorrhagic mass which had not invaded the underlying muscle or overlying skin. It had a yellowish-green pellet of methylcholanthrene embedded superficially. Although a discoloration of the tissue at site IV was the only change noted grossly, a piece was excised for microscopic study. Gross metastases were evident in the right inguinal lymph node, the lungs, the kidneys, and the liver. The lungs were a mass of tapioca-like metastases (Fig. 7). The cortical areas of both kidneys were largely replaced by tumor.

On microscopic examination, tumor A was found to be a mixed-cell sarcoma (Fig. 8) with moderate whorl formation; tumor B, a densely cellular, soft-tissue sarcoma (Fig. 9); tumor C, a spindle-cell sarcoma of the large-cell type (Fig. 10); and tumor D, a loosely constructed mixed-cell sarcoma (Fig. 11). An examination of the sections prepared from the lymph nodes and internal organs revealed extensive replacement by metastatic sarcomatous tissue of the right inguinal lymph node (Fig. 12); of the lungs (extent of replacement shown in Fig. 13); of the kidneys (the predominantly mixed-cell character of the tissue is shown in Fig. 14, the extensive replacement of the cortical tissue by tumor and the accompanying necrosis in Fig. 15, and the type of cell present in the metastatic nodules in both kidneys in Fig. 16); and of the liver (metastatic focus shown in Fig. 17).

An attempt to establish a transplantable sarcoma in cottontail rabbits was made by using sarcomatous tissue from this rabbit. The materials for transplantation were obtained from the primary tumor B and from metastases in the left kidney. From each a piece of tissue approximately 1 cm. in diameter was excised and treated individually in preparation for transplantation according to the technic outlined. The recipients for the transplants were both local and western cottontail rabbits. Twelve rabbits were grouped such that 6 received tissue from the primary tumor and 6 from the metastases. Of the 6 rabbits receiving primary tumor, 2 yielded evidence that the transplanted cells had multiplied. In one animal, rabbit No. 7,

DESCRIPTION OF FIGURES 1 TO 7

All of the specimens were stained with hematoxylin and eosin. The photographs were made by Mr. Merwyn C. Orser.

FIG. 1.—Rabbit No. 1. Epidermoid carcinoma in subcutaneous tissue of right axillary region. The tumor was firmly attached to the overlying skin and was invading the underlying muscle. Mag. × 150.

FIG. 2.—Rabbit No. 2, tumor D. Mixed-cell sarcoma invading muscle. Mag. × 120.

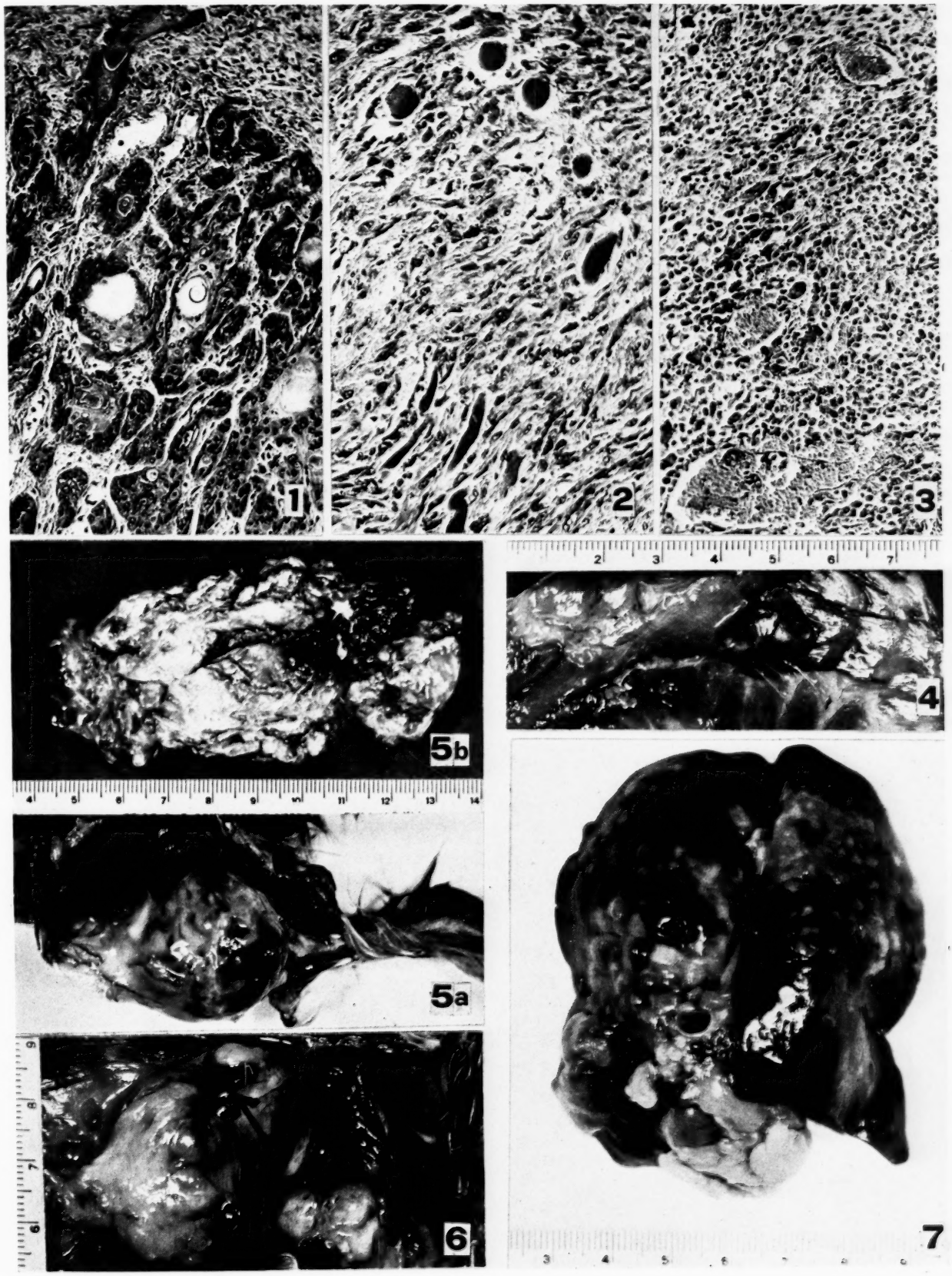
FIG. 3.—Rabbit No. 2. A metastasis in the left inguinal node, composed of both mixed-cell and large spindle-cell varieties of sarcomatous tissue. Note the great vascularity.

FIG. 4.—Rabbit No. 4, site I. "Pellet" of methylcholanthrene in tricaprylin lying superficially in subcutaneous tissue.

FIG. 5.—Rabbit No. 4, tumor B. A highly cellular primary sarcoma infiltrating and replacing muscular tissue. (a) The tumor *in situ*. (b) The tumor dissected out of the surrounding tissue.

FIG. 6.—Rabbit No. 4, tumor C. A loose mixed-cell sarcoma. A "pellet" of methylcholanthrene is indicated by the arrow.

FIG. 7.—Rabbit No. 4. The lungs largely replaced by tapioca-like metastases.



FIGS. 1-7

nodular swellings between 1.0 and 1.5 cm. in diameter were noticed on the tenth day following transplantation. Although these tumors increased in size to from 2.5 to 3.0 cm. in diameter by the 16th day, by the 23rd day complete regression had taken place. Further attempts at transplantation were therefore impossible. On the other animal, rabbit No. 8, giving evidence that the transplanted cells had multiplied, a small tumor was found at each of two intramuscular sites 10 days after inoculation. One of these tumors was excised for passage; the other regressed during the following 10 days. Passage was made to each of 10 cottontails, 5 western and 5 local. None gave evidence that the transplanted cells survived.

In the second group (the group that received tissue derived from the kidney metastases), a single rabbit, rabbit No. 9, yielded evidence that the transplanted cells had grown. Tumors were noted on this recipient at all 6 sites of injection 33 days after the transplants were made. They grew rapidly and infiltrated the contiguous tissues. By the 45th day after injection, the size of the transplants ranged from $0.8 \times 0.8 \times 0.6$ cm. to $2 \times 4.5 \times 2$ cm. Because the transplanted tumors in the 2 preceding rabbits, rabbit No. 7 and rabbit No. 8, had regressed so rapidly, all the actively growing tumors were removed from rabbit No. 9 for transplantation and passed to 18 cottontail rabbits, each tumor being treated individually in preparation for transfer to each of 3 cottontails. Only 1 of the 18 recipients (examined weekly for 98 days) showed any evidence that the tissue transplants had become established. In this animal, rabbit No. 10, definite growths were present 10 days after injection at 2 of the 6 sites, but they had completely regressed 10 days later.

Rabbit No. 5.—Rabbit No. 5 was injected on June 13, 1939. On the 63rd day after injection, single, small, discrete, shot-like nodules were present at sites III and IV. By the 175th day, all 4 sites of inoculation presented ill-defined, nonindurated tumors that merged into the surrounding tissues. Four weeks later, on the 203rd day, tumor A, having grown rapidly in the preceding 3 weeks, had become an irregular indurated mass measuring approximately $4 \times 9.5 \times 6$ cm., whereas tumors B, C, and D were essentially unchanged. Tumor A continued to grow rapidly. Eight months after injection, on the 245th day, it measured $6 \times 12 \times 7$ cm., was resilient to palpation, and had diffusely infiltrated the neighboring tissues. Tumors B, C, and D, on the other hand, showed no change. The animal died 10 days later, on the 255th day. Following removal of the overlying skin, tumor A was photographed *in situ* (Fig. 18a) and in hemi-section after it had been dissected out of the surrounding tissues (Fig. 18b). It was a huge tumor that encircled the shoulder girdle, weighed 215 gm., and measured $7 \times 13 \times 7$ cm. Hemi-section of the tumor showed it to be myxosarcomatous in consistency. The other 3 tumors were ovoid in shape and measured approximately 2 cm. in diameter. Metastases were not evident grossly. On microscopic examination it was found that all 4 tumors were mixed-cell sarcomas, loosely constructed, vascular, and edematous. The cellular type of reaction is shown in photomicrographs of tumor A (Fig. 19) and Tumor B (Fig. 20). Soon after this animal's death, pieces of tissue were excised from the interior of tumor A for transfer to each of 12 cottontail rabbits. All these recipients died within 36 hours from *Pasteurella cuniculicida* infection transmitted with the fragments of tumor.

Rabbit No. 6.—Rabbit No. 6 was injected on May 16, 1939. The 4 sites of injection were diffusely indurated 28 days later. After another month, nodules measuring from 1 to 2 cm. in diameter had developed at each site. During the next 5 months (210th day) the nodules became slightly larger. They were circumscribed, soft, and readily movable. Within the next several weeks (242nd day) further changes were limited to tumor A, which enlarged to approximately $1 \times 2 \times 2$ cm. Thereafter changes occurred more rapidly. By the 270th day, tumor A had enlarged to $2.5 \times 2.5 \times 3$ cm.; its upper surface had softened, but the basilar portion was hard, nodular, and firmly fixed to the underlying tissues. Tumor B, measuring 2.5 cm. in diameter, was hard and nodular; tumor C was soft, amorphous, and measured $2.5 \times 3.0 \times 1.5$ cm.; the tissues at site IV were unchanged. Three weeks later (294th day), tumors were present at all 4 sites of injection. Each had infiltrated contiguous tissues. The left axillary node was enlarged but not indurated. The cachectic animal was exsanguinated to make available tumor tissue for transfer.

At necropsy the skin overlying the tumors was neither broken nor attached. Tumor A, measuring $3 \times 4 \times 3.5$ cm., and tumor B, measuring $2.5 \times 3.5 \times 3.5$ cm., were soft and showed hemorrhagic necrotization. The regional lymph nodes were enlarged and soft. Tumor C, measuring $1 \times 1.5 \times 1$ cm., was firm. No metastases or other abnormalities were found grossly.

On microscopic examination it was found that tumor A was a soft-tissue sarcoma in which were incorporated areas of spindle-cell (large type) and mixed-cell varieties of tissue. Many of the cells were undergoing mitosis. Sections of the extensively vascularized tumor showed that a moderate number of red blood cells had extravasated into the tumor. Areas of necrotization interspersed amongst what appeared to be actively growing tissue were accompanied by a moderately inflammatory reaction. While tumor B showed essentially the same histologic picture as tumor A, less necrotization and more extensive infiltration with replacement of the surrounding tissues had taken place. Some of the invaded muscle tissue had undergone hyaline degeneration. Tumor C consisted of an epidermoid carcinoma mounted in a much larger mixed-cell sarcoma. The two types of tumor tissue were merged at the periphery of each tumor. The intact overlying epithelium gave no evidence to suggest that the carcinogenic agent had been in direct contact with the epithelial cells. Tumor D was a highly vascularized spindle-cell sarcoma.

Transplantation of a tumor from rabbit No. 6 was attempted. Tumor B was selected as the source of material because of its firmness and the absence of gross degenerative or inflammatory changes. A portion of the tumor was inoculated into 12

DESCRIPTION OF FIGURES 8 TO 14

FIG. 8.—Rabbit No. 4, tumor A. Primary mixed-cell sarcoma with a tendency to whorl formation. Mag. $\times$ 120.

FIG. 9.—Rabbit No. 4, tumor B. Primary highly cellular sarcoma invading the muscle. Mag. $\times$ 120.

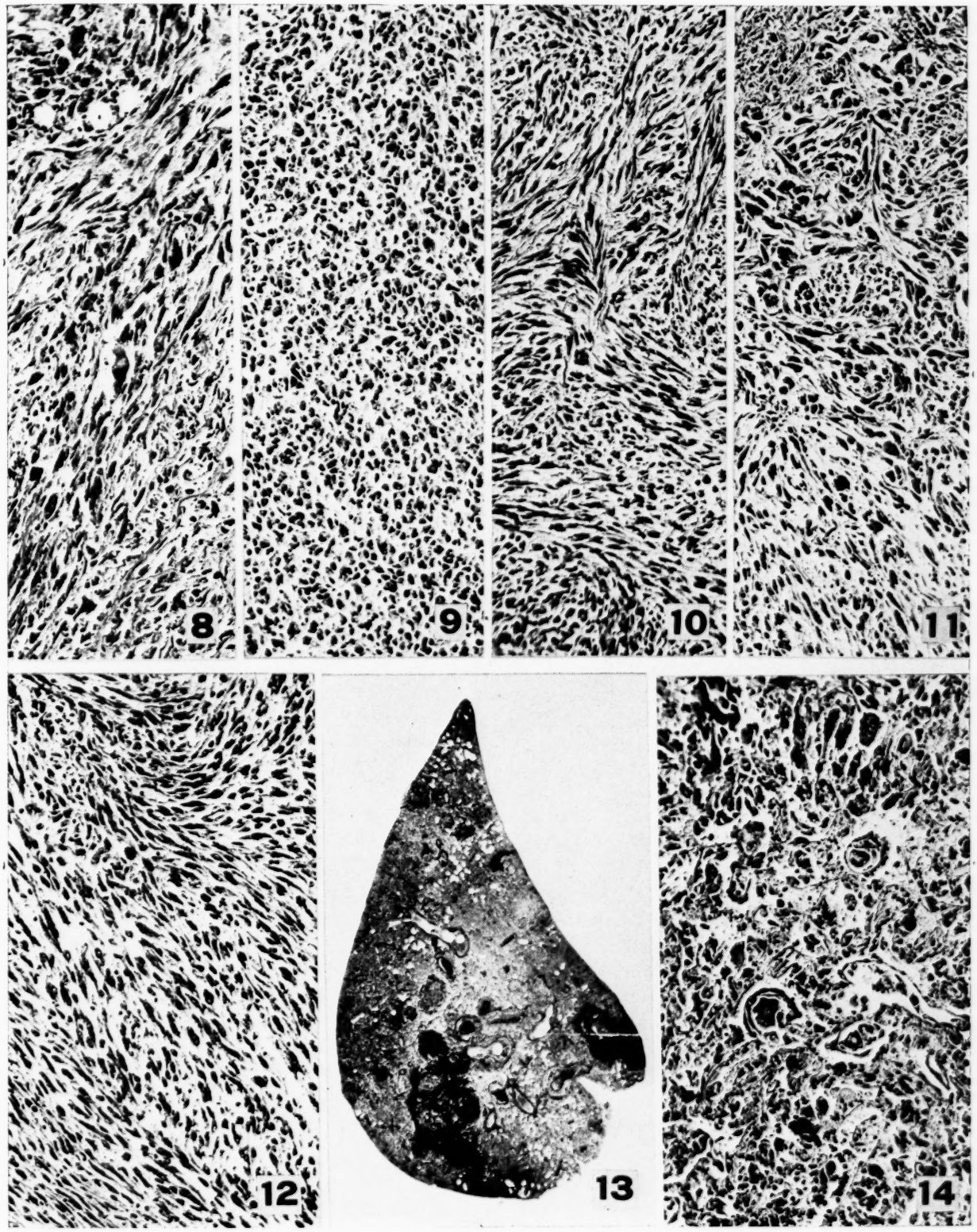
FIG. 10.—Rabbit No. 4, tumor C. Primary spindle-cell sarcoma. Mag. $\times$ 120.

FIG. 11.—Rabbit No. 4, tumor D. Primary mixed-cell sarcoma. Mag. $\times$ 120.

FIG. 12.—Rabbit No. 4. Metastasis in right inguinal lymph node. Mag. $\times$ 120.

FIG. 13.—Rabbit No. 4. Lung in section showing extensive replacement by metastatic sarcomatous tissue. Mag. $\times$ 3.

FIG. 14.—Rabbit No. 4. Metastatic mixed-cell variety of sarcomatous tissue in the lung. Mag. $\times$ 120.



FIGS. 8-14

cottontail rabbits, 6 local and 6 western. None of the recipients gave evidence within 6 months that the transplanted cells had grown.

SUMMARY AND DISCUSSION

The experiments described in the present paper have demonstrated that methylcholanthrene in tricaprylin is an efficacious carcinogenic agent in the cottontail rabbit,[4] a new host for this type of work. Sarcomas, carcinomas, or both, resulted in all 6 rabbits under study at one or more of the sites of subcutaneous or intramuscular injection in from 176 to 295 days following the introduction of the carcinogen. The results of these experiments have been summarized in Table I. It may be seen that tumors developed at only a single site of injection in 2 animals, at all 4 sites in the rest. Sarcomatous metastases to a regional lymph node occurred in 2 rabbits; to the lungs, liver, and kidneys in addition in a single animal.

It is unusual for the cells of other experimental sarcomas to metastasize to lymph nodes, yet a regional node in each of 2 rabbits was found to be largely replaced by sarcomatous tissue. Since the cellular characteristics of these metastases were the same as those of the primary tumor, one may assume that tumor cells rather than methylcholanthrene had passed through the lymphatics to the regional nodes. Possibly the carcinogenic agent had influenced the regional lymphatics in a way to favor metastasis, for metastases were found only in the regional nodes.

Although each of the rabbits under study was permitted to live out its natural life, the death of but a single rabbit could be directly attributed to the uncomplicated malignancy, and this animal had the most extensive metastases. The other 5 rabbits died of intercurrent infection or injury. The extensive degenerative changes in the primary lesions favored the invasion of bacteria.

The attempts to transmit the methylcholanthrene-induced tumors by serial passage in cottontail rabbits met with very little success. Of the 64 rabbits used as recipients for sarcoma tissue, only 3 yielded any evidence that the transplants had grown. In 2 of these rabbits, the transplants regressed within 10 days, but in the third rabbit the transplanted tissue grew rapidly to a size that permitted further passage to 18 cottontails. In but a single animal of this group, however, was there any evidence that the transplanted cells had multiplied, and even in this rabbit the resultant tumor regressed promptly. Nowhere in the course of these transmission experiments were findings encountered which suggested that a filterable virus was playing any part in the reaction.

Although the rabbit has many obvious advantages—its size, longevity, ready immunizability, etc.,—over the mouse and the rat as a host in which to investigate experimental cancer, it has been but little used, for a uniformly effective carcinogenic agent has not been recognized. The results of the present study suggest that such an agent (methylcholanthrene in tricaprylin) has now been found, for sarcomas and carcinomas were elicited in from 176 to 295 days in all of the rabbits that survived intercurrent infection or injury. It should be understood that these time intervals do not indicate when the tumor appeared, but merely the number of days between injection and the animal's death, at which time the histopathological diagnosis was made. This procedure was adopted to avoid all manipulations that might interfere in the natural course of the host's reaction to the carcinogenic agent. Obviously, a diagnosis could have been made much earlier.

Fieser (5) has emphasized the importance in experimental cancer research of avoiding the use of solvent media of varying or unknown composition. He suggested that any of several of the glycerides of the normal saturated fatty acids, of which tricaprylin is a good example, be employed. Accordingly, tricaprylin was used in the present study and was found to be a most satisfactory medium for the parenteral introduction of methylcholanthrene. It is possible, therefore, to limit the materials introduced into a single host to two chemicals of known purity and composition, methylcholanthrene and tricaprylin.

Finally, the present observations show further that methylcholanthrene is one of the most efficacious of the carcinogenic agents, and that a new host, the cottontail rabbit, genus *Sylvilagus,* is available for experimental cancer research.

DESCRIPTION OF FIGURES 15 to 20

FIG. 15.—Rabbit No. 4. Metastases to kidney with extensive replacement of cortical tissue by sarcomatous tissue of mixed-cell type. Some of the tissue has necrotized. Mag. × 3.

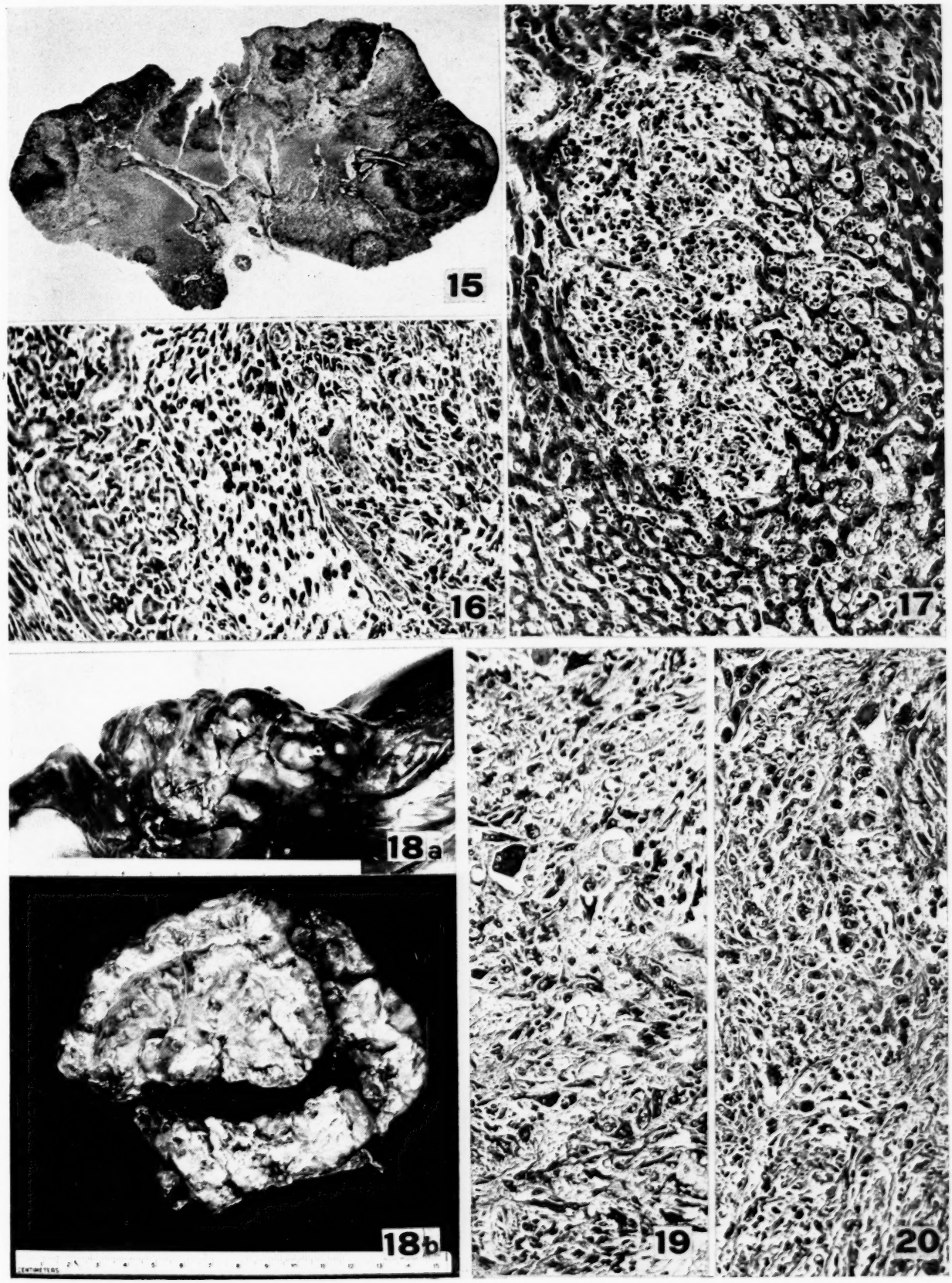
FIG. 16.—Rabbit No. 4. Cellular type of the kidney metastases. Mag. × 120.

FIG. 17.—Rabbit No. 4. Metastatic focus in liver. Mag. × 120.

FIG. 18.—Rabbit No. 5, tumor A. (a) A nodular, resilient, primary mixed-cell sarcoma photographed *in situ.* (b) The same tumor after hemi-section following dissection from the encircled shoulder girdle. Note the loose construction and myxomatoid character.

FIG. 19.—Rabbit No. 5, tumor A. A primary mixed-cell sarcoma.

FIG. 20.—Rabbit No. 5, tumor C. A primary mixed-cell sarcoma.



FIGS. 15-20

CONCLUSION

Methylcholanthrene is an effective carcinogenic agent in the cottontail rabbit, genus *Sylvilagus,* a host which has not previously been used for experimental cancer research. All 6 of the rabbits under study that survived for 175 days or more developed sarcomas, carcinomas, or both, within 267 days after receiving the carcinogenic hydrocarbon. Tricaprylin, a glyceride of a normal saturated fatty acid, was found to be a satisfactory vehicle in which to incorporate the methylcholanthrene.

REFERENCES

1. Andrews, C. H., and Ahlström, C. G. A Transplantable Sarcoma Occurring in a Rabbit Inoculated with Tar and Infectious Fibroma Virus. J. Path. & Bact., **47**:87-99. 1938.
2. Andrews, C. H., Ahlström, C. G., Foulds, L., and Gye, W. E. Reaction of Tarred Rabbits to the Infectious Fibroma Virus (Shope). Lancet, **2**:893-895. 1937.
3. Burrows, H., and Boyland, E. Neoplasia in Rabbits Following the Administration of 1:2:5:6-Dibenzanthracene. Am. J. Cancer, **32**:367-382. 1938.
4. Cook, J. W., and Kennaway, E. L. Chemical Compounds as Carcinogenic Agents. First Supplementary Report: Literature of 1937. Am. J. Cancer, **33**:50-97. 1938.
5. Fieser, L. F. Carcinogenic Activity, Structure, and Chemical Reactivity of Polynuclear Aromatic Hydrocarbons. Am. J. Cancer, **34**:37-124. 1938.
6. Kidd, J. G., Beard, J. W., and Rous, P. Serological Reactions with a Virus Causing Rabbit Papillomas Which Become Cancerous. I. Tests of the Blood of Animals Carrying the Papilloma. J. Exper. Med., **64**:63-77. 1936.
7. Kidd, J. G., and Rous, P. Effect of the Papilloma Virus (Shope) upon the Tar Warts of Rabbits. Proc. Soc. Exper. Biol. & Med., **37**:518-520. 1937.
8. Kidd, J. G., and Rous, P. The Carcinogenic Effect of a Papilloma Virus on the Tarred Skin of Rabbits. II. Major Factors Determining the Phenomenon: The Manifold Effects of Tarring. J. Exper. Med., **68**:529-562. 1938.
9. Kidd, J. G., and Rous, P. Cancers Deriving from the Virus Papillomas of Wild Rabbits under Natural Conditions. J. Exper. Med., **71**:469-494. 1940.
10. Klinke, J. Die carcinogene Wirkung des 1.2-Benzpyrens am Kaninchen. Ztschr. f. Krebsforsch., **46**:334-342. 1937.
11. Lacassagne, A., and Nyka, W. Faible réaction, à l'injection intraveineuse du virus de Shope, au niveau des papillomes obtenus par badigeonnages au benzopyrène chez des lapins à hypophyse détruite. Bull. Assoc. franç. p. l'étude du cancer, **26**:154-162. 1937.
12. Rous, P. The Virus Tumors and the Tumor Problem. Am. J. Cancer, **28**:233-272. 1936.
13. Rous, P., and Beard, J. W. The Progression to Carcinoma of Virus-Induced Rabbit Papillomas (Shope). J. Exper. Med., **62**:523-548. 1935.
14. Rous, P., and Kidd, J. G. The Carcinogenic Effect of a Virus upon Tarred Skin. Science, **83**:468-469. 1936.
15. Rous, P., and Kidd, J. G. The Carcinogenic Effect of a Papilloma Virus on the Tarred Skin of Rabbits. I. Description of the Phenomenon. J. Exper. Med., **67**:399-428. 1938.
16. Schürch, O. Zur krebserzeugenden Wirkung des Benzpyren beim Kaninchen. Ztschr. f. Krebsforsch., **49**:353-362. 1939-40.
17. Seelig, M. G., and Cooper, Z. K. A Review of the Recent Literature of Tar Cancer (1927-1931 Inclusive). Am. J. Cancer, **17**:589-667. 1933.
18. Shope, R. E. Infectious Papillomatosis of Rabbits. J. Exper. Med., **58**:607-624. 1933.
19. Syverton, J. T. The Papilloma-Carcinoma Sequence in the Cottontail (*Sylvilagus*) Rabbit. Third International Congress for Microbiology, Abstracts of Communications, New York, Section III, 341-342. 1939.
20. Syverton, J. T., and Berry, G. P. Carcinoma in the Cottontail Rabbit Following Spontaneous Virus Papilloma (Shope). Proc. Soc. Exper. Biol. & Med., **33**:399-400. 1935.
21. Syverton, J. T., and Berry, G. P. Sarcomata and Carcinomata Induced in Cottontail Rabbits by Methylcholanthrene. Science, **91**:298. 1940.
22. Woglom, W. H. Experimental Tar Cancer. Arch. Path., **2**:533-576 and 709-752. 1926.

Abstracts

Reports of Experimental Research

Hormones

FEKETE, E., WOOLLEY, G., and LITTLE, C. C. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine] HISTOLOGICAL CHANGES FOLLOWING OVARIECTOMY IN MICE. I. DBA HIGH TUMOR STRAIN. J. Exper. Med., 74:1-8. 1941.

Female mice of the Dba strain were ovariectomized at birth and then killed at different ages. It was found that the vagina, uterus, and mammary glands showed a gradual recovery from the castrate state and reached the stage which they presumably can attain only under the influence of estrogenic hormones. Between the ages of 14 and 28 months, 37 out of 75 animals examined developed tumors of the mammary gland. These were 3 adenomas and 34 carcinomas. The suggestion is made that estrogen originated in some other organ, as ovarian regeneration had not taken place. The suprarenal cortex is considered as the possible source of the estrogenic hormones because of the nodular hyperplasia that consistently developed in these organs following ovariectomy.—D. S.

LIPSCHÜTZ, A., and VARGAS, L., JR. [Dept. of Exper. Med., National Health Service, Republic of Chile, Santiago, Chile] INFLUENCE OF AGE ON SEROSAL TUMORAL REACTION OF THE GUINEA PIG TOWARDS ESTROGENS. Proc. Soc. Exper. Biol. & Med., 48:584-587. 1941.

"Aged" castrate female guinea pigs (700 to 1050 gm.) and "young" animals (180 to 245 gm.) were given subcutaneous injections of 80 γ of estradiol monobenzoate in oily solution. Injections were made 3 times weekly for 2½ to 3 months.

Uterine and extragenital fibroids were obtained in both groups. The incidence of abdominal fibroids in the young animals was no less than in the older ones. Whether age may influence the size and number of fibroids is still to be determined.—M. B.

LIPSCHÜTZ, A., VARGAS, L., JR., and NUNEZ, C. [Dept. Exper. Med., National Health Service, Republic of Chile, Santiago, Chile] COMPARATIVE ANTITUMORAL ACTION OF DESOXYCORTICOSTERONE ACETATE AND TESTOSTERONE PROPIONATE. Proc. Soc. Exper. Biol. & Med., 48:271-274. 1941.

Tablets of estradiol (7.5 to 27.0 mgm.) were implanted subcutaneously in 18 castrate female guinea pigs (250 to 320 gm.). Simultaneously tablets of testosterone propionate (17.0 to 32.0 mgm.) were implanted on the opposite side of the same animals. They were sacrificed 55 and 56 days later. Five animals out of 18 showed uterine tumors and 6 animals also had extragenital tumors.

The tablets were removed from their fibrous envelope, kept for 20 hours at 60° C. and weighed. The average absorption of estradiol was 2.5 mgm., that of testosterone propionate 11.9 mgm.

Desoxycorticosterone acetate is thus quantitatively more efficient than testosterone in preventing tumor formation in the guinea pig (by comparison with previous experiments). It is also more efficient than testosterone in antagonizing other effects of estradiol such as opening of the vagina and increase of uterine weight.—M. B.

Viruses

BLOCH, H. [Hyg. Inst. der Univ. Basel, Switzerland] EXPERIMENTELLE UNTERSUCHUNGEN ÜBER BEZIEHUNGEN ZWISCHEN BAKTERIOPHAGEN UND MALIGNEN TUMOREN. [EXPERIMENTAL INVESTIGATIONS ON THE RELATION BETWEEN BACTERIOPHAGE AND MALIGNANT TUMORS.] Arch. f. d. ges. Virusforsch., 1:481-496. 1939-1940.

Since there are morphological and physical chemical similarities between various types of viruses and bacteriophage, the investigation was undertaken to determine whether the relation between malignant tumor and bacteriophage is similar to that of malignant tumor to viruses. It was shown that, as in the case of many viruses, there is a concentration of the bacteriophage in the malignant tumor. The bacteriophage is found to remain in the malignant tumor (Ehrlich's mouse carcinoma, Brown-Pearce rabbit carcinoma, Rous sarcoma) longer than in the normal organs of the animal concerned. This occurs either on direct injection of the lysin into the tumor or on introducing it into the organism subcutaneously or intravenously. If the lysin circulates in the blood, it accumulates selectively in the tumor. It was found that the Ehrlich carcinoma loses its transplantability in a high percentage of cases because of its bacteriophage content. The bacteriophage shows a similar predilection for inflammatory or quickly growing testicular tissue as it shows for neoplastic tissue. Coliphage S was used for these investigations.—B. N. H.

DURAN-REYNALS, F. [Dept. of Bacteriology, Yale Univ. Med. Sch., New Haven, Conn.] THE RECIPROCAL INFECTION OF DUCKS AND CHICKENS WITH TUMOR-INDUCING VIRUSES. Cancer Research, 2:343-369. 1942.

The intravenous injection of newborn ducks with the virus of the Rous sarcoma results in the development of either immediate or late tumors. The former, developed within the first 30 days after injection, showed the characteristics of the sarcoma growing in chickens and were transmissible to chickens but not to ducks. The latter were found several months after injection, consisted of different sarcomas and lymphoblastomas, and were transmissible to ducks but not to chickens. In the former case the chicken virus did not vary, and the duck disease

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was the result of heterologous infection; in the latter case, the virus varied, and the disease was the result of an homologous infection. The same virus variation was obtained by passages of Rous tumor cells through young ducks. In this case a gradual transformation from the chicken strain into a duck strain took place.

Reversing the above sequence, chicks injected with the duck variants developed either immediate or late lesions. Characteristically, the former consisted of sarcomas arising from bones, much as in the disease of ducks, and were serially transmissible through ducks but not through chickens; the latter consisted of different types of leukoses and the condition known as osteopetrosis, transmissible, although with difficulty, through chickens but not through ducks. Therefore, as in the chicken virus-duck sequence, these two sorts of tumors developed as a result of either homologous or heterologous infections. There was no reversion to the original Rous tumor.—Author's abstract.

GAVRILOV, W., and FESTER, A. [Inst. Hyg., Province d'Anvers, France] QUELQUES PROPRIÉTÉS DU VIRUS DE SARCOME ROUS. [SOME PROPERTIES OF THE ROUS SARCOMA VIRUS.] Arch. f. d. ges. Virusforsch., 1:104-113. 1939-1940.

The fluid of a Rous sarcoma culture (hanging-drop method), on inoculation into a Leghorn chicken, produced a tumor in 15 days. Upon repeated passages in Leghorn chickens, there were 60% takes on the first inoculation and on the second 63%. The use of grafts produced tumors within 15 to 20 days, the number of takes being greater than when the culture broth was used. When the tumor filtrate was used, 40% positive takes resulted.

The sarcoma, when observed under low power, may be divided into 4 zones: 1. central, which is practically always necrotic; 2. second, which is formed of cells more dense than the first zone with vessels full of erythrocytes; 3. third, which is characterized by an aggregate of fusiform cells with clear nuclei containing one or two nucleoli; 4. fourth, which is represented by islets of rounded cells with round nuclei and one or two nucleoli.

The immediate result of the injection of the filtered or unfiltered virus is a histiocytic reaction which in the presence of the virus can develop into a real tumor. The histiocytes undergo an influence, probably due to the virus which divides them into two groups: (a) fusiform cells forming rods and (b) round cells with clear nuclei which give rise to a real tumor. The authors believe that the tumors are produced by a biological agent which must have living cells for its multiplication and believe that histiocytes must be present for the development of the virus.—B. N. H.

Genetics

FIGGE, F. H. J., STRONG, L. C., STRONG, L. C., JR., and SHANBROM, A. [Yale Univ. Sch. of Med., New Haven, Conn.] FLUORESCENT PORPHYRINS IN HARDERIAN GLANDS AND SUSCEPTIBILITY TO SPONTANEOUS MAMMARY CARCINOMA IN MICE. Cancer Research, 2:335-342. 1942.

The finding is reported that mice of strains with high susceptibility to breast cancer show more intense red porphyrin fluorescence of the Harderian glands than do mice belonging to strains with low susceptibility to cancer. The close parallelism between the degree of red fluorescence of the Harderian gland and the degree of cancer susceptibility in over 750 mice of 16 different strains is regarded as evidence in favor of the following hypothesis: There is a direct or indirect relationship between porphyrin metabolism and the factors that determine cancer susceptibility. In addition to the above-mentioned strain-limited difference in porphyrin fluorescence, there are also age and sex differences. The desirability of the introduction of the porphyrin concept into the already complex situation of cancer susceptibility in mice is discussed.—Authors' abstract.

GREENE, H. S. N. [Rockefeller Inst. for Med. Research, Princeton, N. J.] UTERINE ADENOMATA IN THE RABBIT. III. SUSCEPTIBILITY AS A FUNCTION OF CONSTITUTIONAL FACTORS. J. Exper. Med., 73:273-292. 1941.

An investigation of 849 rabbits over 2 years of age showed that 142 or 16.7% developed uterine adenomas. All the tumors originated in the endometrium of the uterine fundus and no instance of cervical cancer was seen. The incidence varied widely in relation to age, breed, and genetic constitution and there was a close parallelism between the incidence of toxemia of pregnancy and uterine tumors. The position of different genetic groups in a susceptibility scale was identical with respect to both disorders. The author suggests that this association may be the result of liver damage incident to toxemia. Impairment of this organ may result in its inability to inactivate estrin, which may rise to a carcinogenic level in the blood stream.—D. S.

GROSS, L. [Christ Hosp., Cincinnati, Ohio] INFLUENCE OF SEX ON RESISTANCE TO INTRAPERITONEAL INOCULATION OF SARCOMA IN MICE. Proc. Soc. Exper. Biol. & Med., 49:67-71. 1942.

Groups of male and female mice, 2 to 3 months old, of the albino E. S. strain were inoculated intraperitoneally with equal doses of sarcoma S37.

Following inoculation with small doses, 89 of 101 males (88%) developed tumors as compared with 65 of 110 females (59%). This difference in resistance between the sexes was completely abolished by larger doses of the tumor (males 94%, females 95%). The tumors produced by intraperitoneal inoculation of the sarcoma were uniformly fatal and no instance of spontaneous regression was observed.—M. B.

STRONG, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] THE TRANSPLANTATION OF AN ADENOCARCINOMA OF THE PREPUTIAL GLAND IN MICE OF THE A STRAIN. Cancer Research, 2:332-334. 1942.

The observation has been made of the sporadic occurrence of an adenocarcinoma of the preputial gland in a mouse of the A strain. This finding emphasizes, therefore, a class of neoplasia in mice in which no tendency to transmit the tumor could be detected. This noninherited class now includes melanoma, lymphoblastoma, small round cell sarcoma, squamous carcinoma of the anus, certain cases of carcinoma of the mammary gland, and adenocarcinoma of the preputial gland. The rare type of spontaneous tumor occurring in a mouse of a highly inbred strain will grow subcutaneously in all mice inoculated even though the tendency to give rise to the spontaneous tumor is not inherited, thus emphasizing the

idea that the mechanism involved in the transplantation of neoplastic tissue is unquestionably entirely independent of the mechanism that determines the origin and nature of the spontaneous tumors in mice.—Author's abstract.

VISSCHER, M. B., GREEN, R. G., and BITTNER, J. J. [Depts. of Physiology and Bacteriology, Univ. of Minnesota, Minneapolis, Minn., and the Jackson Memorial Lab., Bar Harbor, Maine] CHARACTERIZATION OF MILK INFLUENCE IN SPONTANEOUS MAMMARY CARCINOMA. Proc. Soc. Exper. Biol. & Med., 49:94-96. 1942.

Fostered female C3H mice, 1 month old, were used as test animals. Lactating breast tissue was obtained from high cancer C3H and A strain mice. The material was at once homogenized by addition of between one and two times its volume of sterile distilled water and the resulting fluid centrifuged at 15,000 r.p.m. for 30 minutes. Three layers resulted: a fatty top layer (the fat fraction), a middle aqueous layer (the first supernatant fluid), and a bulky precipitate (the first sediment). The fat fraction and first supernatant were removed, and the latter spun at 40,000 r.p.m. for 60 minutes, yielding a final supernatant fluid and the second sediment.

Homogenized tissue and most of the above fractions were given either by subcutaneous injection or stomach tube. At 12 months of age the incidence of verified mammary carcinoma in the mice following administration of the above substances was homogenized tissue, 71%; second sediment, 55%; first sediment, 41%; fat fraction, 14%; and final supernatant, 0%.

The precise nature of the active agent is not determined but probably is a colloid of high molecular weight and may be a virus.—M. B.

Physical Factors

BISCHOFF, F. [Chemical Lab., Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.] CONDITIONS REQUIRED TO PRODUCE A PROLONGED HYPOTHERMIA IN THE MOUSE. Cancer Research, 2:370 371. 1942.

The rectal temperature of mice, which are maintained at an environmental temperature of 5 to 7° C. and which have access to an adequate supply of foodstuffs, does not fall immediately to temperatures below 33° C. but may be maintained in the lower limits of the normal range for 24 to 48 hours. This adjustment occurs at expense of endogenous calorie-supplying depots and by means of increase (about double) in caloric intake. The influence of these experimental conditions upon tumor growth should be interpreted on the basis of a profound increase in metabolism, without losing sight of the derangement of the metabolic and catabolic processes which obviously ensue. To ascertain the immediate effects of hypothermia on tumor growth the period of increased metabolism can be reduced to 2 hours or less by using an environmental temperature −2 to −5° C., entailing a loss of body heat which exceeds the capacity for adjustment.—Author's abstract.

Biochemistry and Nutrition—Chemotherapy

SWYER, G. I. M. [Dept. of Human Anatomy, Oxford Univ., Oxford, England] THE CHOLESTEROL CONTENT OF NORMAL AND ENLARGED PROSTATES. Cancer Research, 2: 372-375. 1942.

The Schultz reaction for cholesterol suggests that the adenomatous zones of enlarged prostates contain a high concentration of cholesterol, and that the cholesterol concentration of enlarged prostates is higher than that of normal prostates. These observations were confirmed by quantitative determinations.

The "adenomas" of enlarged prostates contain, on the average, twice as high a concentration of cholesterol as the remainder of the prostate.—Author's summary.

WEST, P. M., and WOGLOM, W. H. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] ABNORMALITIES IN THE DISTRIBUTION OF BIOTIN IN CERTAIN TUMORS AND EMBRYO TISSUES. Cancer Research, 2:324-331. 1942.

With but one exception all malignant tumors studied, whether of animal or human origin, differed widely in their biotin content from the corresponding normal tissues.

The biotin levels of embryo tissues also diverged widely from those of normal adult tissues, the major shift from embryonic to adult biotin values occurring in the rat within 3 days after birth.

In all cases, the biotin content of both tumor and embryo tissues deviated in the same direction from the corresponding normal adult levels.

Attempts to raise the general biotin content of animals by injection of biotin concentrate were unsatisfactory because of rapid excretion of the excess vitamin.

Both tumors and normal tissues could be greatly depleted of biotin by maintaining the animals on a ration containing avidin, and although the animals suffered severe deficiency symptoms as a result, the growth of certain transplanted tumors was unimpaired. Thus, while biotin is known to be an essential factor in the metabolism of normal mammalian tissues, these tumors, at least, are capable of maintaining normal activity in its relative absence.—Authors' summary.

Immunology

FURTH, J., and KABAT, E. A. [Cornell Univ. Med. College, New York, N. Y.] IMMUNOLOGICAL SPECIFICITY OF MATERIAL SEDIMENTABLE AT HIGH SPEED PRESENT IN NORMAL AND TUMOR TISSUES. J. Exper. Med., 74:247-256. 1941.

Immunological investigations were carried out on materials sedimentable at high speed (27,000 r.p.m.) which were found in all normal and neoplastic tissues studied. The sedimentable material contained the Forssman antigen and the Wassermann hapten. In the chicken and mouse spleen, kidney, lung, and tumor large amounts of Forssman antigen were present while the liver was devoid of this antigen. Also extracts from spleen and kidney of man, beef, and rabbit contained no Forssman antigen. These heavy materials exhibited species, organ, and individual specificity and produced several antibodies which were demonstrated by absorption tests. By complement fixation tests, heavy materials from chicken sarcoma and chicken spleen could not be distinguished. In the light of these observations, the authors suggest that the heavy sedimentable material obtained from different organs is not a single substance but a mixture of substances.—D. S.

KABAT, E. A., and FURTH, J. [Cornell Univ. Med. College, New York, N. Y.] NEUTRALIZATION OF THE AGENT CAUSING LEUKOSIS AND SARCOMA OF FOWLS BY RABBIT ANTISERA. J. Exper. Med., 74:257-261. 1941.

Rabbits were immunized against heavy material obtained from normal chicken spleen and from chicken tumors. The rabbit antiserums against heavy material from the tumor contained neutralizing antibodies while the antiserums against heavy material from normal chicken spleen produced no neutralizing antibodies. However, both materials produced complement-fixing antibodies which are unrelated to the neutralizing antibodies. This was indicated by the observation that an antitumor serum contained neutralizing antibodies but no complement-fixing antibodies. The complement-fixing antibodies, in another antitumor serum, could be removed by absorption with a suspension of cells from normal chicken spleen without any detectable effect on the neutralizing potency of the serum. Also, antiserums against heavy materials from normal spleen did not neutralize the agent but fixed complement in high dilutions.—D. S.

NAFTOLIEV, J. A. [Lab. Expér. de la Clinique Oncologic, Inst. de Physio-Therapie, Sverdlovsk, Russia] IMMUNISATION DE LAPINS D'APRÈS EEZREDKA CONTRE LA TUMEUR MALIGNE DE BROWN-PEARCE. [RABBIT IMMUNIZATION AGAINST BROWN-PEARCE TUMOR.] Bull. biol. et méd. expér. URSS., 9:513-514. 1940.

Thirty-two rabbits were inoculated intradermally with a suspension of Brown-Pearce tumor. Three rabbits died of large, metastasizing growths; one died of empyema; two others developed no tumor but a second inoculation resulted in rapidly growing fatal tumors.

In the remaining 26 rabbits, small tubercle-like tumors appeared at the site of inoculation in 5 to 7 days. These attained the size of a coffee bean to a small nut, then progressively diminished in size and disappeared.

At varying times after recession of these tumors, the rabbits were reinoculated, some intradermally, some testicularly, and others intraperitoneally. No growth was obtained in any animal up to 6 months after inoculation.—M. B.

Transplantation

BRIGGS, R. [Dept. of Zoology, McGill Univ., Montreal, Canada] TRANSPLANTATION OF KIDNEY CARCINOMA FROM ADULT FROGS TO TADPOLES. Cancer Research, 2:309-323. 1942.

The kidney adenocarcinoma of the adult frog (*Rana pipiens*) has been transplanted to a total of 163 tadpoles to determine if the larval environment has any effect on its structure and behavior. The implants developed into typical growing tumors in all of the following sites: (1) subcutaneous tissue of trunk (7 cases); (2) mesenchyme of dorsal tail fin (27 cases); (3) adjacent to the kidney (2 cases); (4) liver (1 case); and (5) body cavity (1 case). One hundred and eighty control tadpoles received implants of normal kidney tissue and developed no tumors.

Tumors in the trunk subcutaneous tissue grew well and in 2 cases survived metamorphosis unaltered.

Tumors in the tail ceased growth and regressed during later stages in the development of the host tadpoles. This regression occurred before the initiation of metamorphic absorption of the tail.

Typical tumors developed in a small number of cases from implants in the liver, body cavity, and adjacent to the kidney. The majority of implants in these sites were resorbed. Six of the hosts which failed to develop a tumor at the implantation site were found to have multiple tumor loci in the kidneys. These occurred only in hosts which had completed metamorphosis. Many of the individual tumor tubules making up the smaller loci showed structural continuity with normal kidney tubules.—Author's abstract.

GREENE, H. S. N. [The Rockefeller Inst. for Med. Research, Princeton, N. J.] HETEROLOGOUS TRANSPLANTATION OF MAMMALIAN TUMORS. I. THE TRANSFER OF RABBIT TUMORS TO ALIEN SPECIES. J. Exper. Med., 73:461-474. 1941.

Transplantation of rabbit carcinomas (uterine cancer, H-31, the breast cancers, T-36 and B-240, and the Brown-Pearce tumor) was attempted to the anterior chamber of the eyes of guinea pigs, swine, goats, and sheep. Before heterologous transfer was tried, the tumors were always carried for one or more generations in the anterior chamber of rabbit's eyes. Tumors H-31, T-36, and B-240 were successfully transplanted to all the animals tried and the H-31 and T-36 tumors were maintained by serial transfer in the guinea pigs and swine. All attempts to transfer the Brown-Pearce tumor to guinea pigs were unsuccessful. The transplanted tumors were similar to those of the natural host, but no evidence of metastases was found. Attempts to transplant tumors obtained at biopsy, from the eye of guinea pigs, to other sites of the body were unsuccessful.—D. S.

GREENE, H. S. N. [The Rockefeller Inst. for Med. Research, Princeton, N. J.] HETEROLOGOUS TRANSPLANTATION OF MAMMALIAN TUMORS. II. THE TRANSFER OF HUMAN TUMORS TO ALIEN SPECIES. J. Exper. Med., 73:475-486. 1941.

Direct transplantation of 11 different human tumors to the anterior chamber of the eyes of guinea pigs and rabbits was attempted. Transplantations of human carcinomas were successful with breast and colon cancers. Results, from the transplantations of cancers of the tongue, cervix, and fundus, were discarded for infection had occurred. The results of the heterologous transplantation of human sarcomas showed that while a retinoblastoma could not be transplanted, a melanotic sarcoma and a fibrosarcoma were successfully transplanted. Also, in the case of the fibrosarcoma, serial passage to a second generation of animals was successfully performed. Histologically, the appearance of the transplants of the carcinomas differed from that of the spontaneous tumors. However, transplants of the sarcomas were morphologically identical with the primary tumors.—D. S.

LUCKÉ, B., and SCHLUMBERGER, H. [Sch. of Med., Univ. of Pennsylvania and The Morris Biological Farm of the Wistar Institute, Philadelphia, Pa.] TRANSPLANTABLE EPITHELIOMAS OF THE LIP AND MOUTH OF CATFISH. I. PATHOLOGY. TRANSPLANTATION TO ANTERIOR CHAMBER OF EYE AND INTO CORNEA. J. Exper. Med., 74:397-408. 1941.

The description, histopathological structure, and course of a naturally occurring epithelioma of the lip and mouth

in the catfish were noted. It was observed that the proliferative stage of the neoplastic process was preceded and accompanied by a vascular reaction. Transplantations into the anterior chamber of the eye or into the cornea of the catfish were successful, while attempts to transplant the tumor to the anterior chamber of the eyes of two other species of fish and to frogs were unsuccessful.—D. S.

Tissue Culture

GRIGORJEW, N. I. [Oncological Inst., and Inst. of Exper. Med., Leningrad, Russia] EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DIE BROWN-PEARCE'SCHE GESCHWULST. [EXPERIMENTAL STUDIES ON THE BROWN-PEARCE TUMOR] Bull. biol. et méd. expér. URSS., 9:510-512. 1940.

Various properties of the Brown-Pearce tumor were studied in tissue culture. The hanging-drop method was employed, with rabbit plasma and chick embryo extract as medium. Vital dyes were used in some experiments; in others the cultures were fixed and serially sectioned.

Under tissue culture conditions, the Brown-Pearce tumor resembled in general growth of celomic epithelium, although the appearance and behavior of some of the cellular elements resembled connective tissue growths.—M. B.

Clinical and Pathological Reports

Heredity

QUILL, L. A., and MARTING, E. C. [Coll. of Med., Univ. of Cincinnati, Cincinnati, Ohio] EPILOIA, REPORT OF CASES. Surgery, 9:581-590. 1941.

The author presents reports of 7 members in 3 generations of one family with this hereditary disorder which is marked by mental deterioration, epilepsy, cutaneous lesions (adenoma sebaceum), "tuberous" sclerotic patches scattered throughout the cerebral cortex and ventricles, superficial filiform papillomas or fibromas especially of the nail beds, benign tumors of many internal viscera, etc. The genealogy of the epiloia family is presented.—A. M.

Therapy—General

HERRMANN, J. B. [Yale Univ. Sch. of Med., New Haven, Conn.] LOW TEMPERATURE THERAPY OF MALIGNANCY. Connecticut M. J., 5:721-726. 1941.

This is a review presented at the 16th Clinical Congress of the Connecticut State Medical Society in September, 1941.

After tracing briefly the development of this form of treating malignant neoplasms, the author discusses some of its physiological effects. In his own work, the author injected animals with small doses of nembutal, morphine, aspirin, or paraldehyde. These doses were so small that they caused no discernible depression. When these animals were subjected to low temperatures, there was a marked drop in body temperature which was not exhibited by undrugged control animals exposed to the same temperatures. Nembutal and paraldehyde were far more toxic in rats at low temperature than at room temperature.

Local cooling results in regression of the lesion largely due to ischemia which is followed by degeneration, necrosis, liquefaction, and absorption. But cold leaves normal tissues unharmed so that there is normal regeneration. After the neoplasm disappears, recurrence is greatly retarded, and when recurrence does take place, the rate of growth is greatly diminished. Cold itself does not destroy neoplastic tissue completely, however, for it has been shown that various types of mouse tumors can be kept at temperatures as low as -70° C. for intervals varying from 1 week to 1½ years, and at the end of that time if thawed and injected into mice, the tumor will grow.

The chief beneficial result of general cooling is the alleviation of pain, but this is only transient. The size of the primary or metastatic lesions in soft tissues is not reduced. The alleviation of pain is due to injury to the cerebral cortex which becomes edematous. Much better relief can be obtained by the judicious use of drugs, by alcohol injection of nerves, and finally by chordotomy. Cooling may cause pneumonia, the formation of thrombi and emboli, and cardiac failure due to myocardial degeneration. The author concludes that the benefits are not commensurate with the dangers and expense involved.—G. De B.

Skin and Subcutaneous Tissues

de NAVASQUEZ, S. [Dept. of Pathology, Guy's Hosp. Med. Sch., London] METASTASISING BASAL CELL CARCINOMA. J. Path. & Bact., 53:437-439. 1941.

Since Willis (*The Spread of Tumours in the Human Body*, J. and A. Churchill, London, 1934) accepted only 3 examples of metastases from basal cell carcinoma, the present case is thought worthy of report because of several distinctive features: 1. the unequivocal appearance of basal cell carcinoma presented by the tumor; 2. widespread blood-borne metastases in bones and lungs; 3. a relatively benign course over many years; 4. the production of leuko-erythroblastic anemia.—A. H.

FRIEDMAN, H. H., and LEDERER, M. [Jewish Hosp., Brooklyn, N. Y.] MELANOBLASTOMA. Am. J. Surg., 55: 88-95. 1942.

Two cases with widespread and unusual sites of metastases are reported.—H. G. W.

Nervous System

EVANS, J. A. [Lahey Clinic, Boston, Mass.] BRAIN TUMOR, STATUS EPILEPTICUS, AND SKULL FRACTURE: CASE REPORT. Lahey Clinic Bull., 2:189-191. 1941.

A case is reported of a 34-year-old farmer diagnosed and treated as idiopathic epilepsy for 8 years. A ventriculogram was normal 6 years before death and an electroencephalogram 11 days before death showed a pattern seldom seen except in epilepsy. No localizing signs were present. A sudden increase in the severity of symptoms was followed by death in 2 weeks. Autopsy revealed a fibrillary astrocytoma involving the floor of the left lateral ventricle and a recent left temporal skull fracture with epidural hematoma.—C. E. D.

HORRAX, G. [Lahey Clinic, Boston, Mass.] A FOLLOW-UP STUDY (2-8 YEARS) ON SOME OF THE UNCOMMON TYPES OF BENIGN BRAIN TUMORS. Lahey Clinic Bull., 2:130-134. 1941.

Four hundred histologically verified brain tumors were seen at the Lahey Clinic from 1932 to 1939. Of these, 218 were classed as surgically removable. Surgical mortality in this group was 12% and 70% were leading useful lives 2 to 8 years after treatment. Forty-eight uncommon tumors were encountered: 13 hemangiomatous cysts, 10 angiomas, 6 craniopharyngiomas, 4 colloid cysts, 3 cholesteatomas, 2 pinealomas, and 10 miscellaneous and unclassified tumors. There were 9 operative deaths in this group and 5 died subsequently. Of the 34 survivors 26 were engaged in useful occupations 2 to 8 years after operation.—C. E. D.

Female Genital Tract

MORTON, D. G. [Univ. of California Hosp., San Francisco, Calif.] EARLY DIAGNOSIS AND PROPER MANAGEMENT IN CERVICAL CANCER. J.A.M.A., 118:271-273. 1942.

In a series of 432 cervix uteri cancers, the 5-year survivals were better than half for the early cases and less than 1/5 for the late cases. The importance of early diagnosis is emphasized.—H. G. W.

ROBERTSON, T. D., HUNTER, W. C., LARSON, C. P., and SNYDER, G. A. C. [Univ. of Oregon Med. Sch., Portland, Oreg.] BENIGN AND MALIGNANT STROMAL ENDOMETRIOSIS. Am. J. Clin. Path., 12:1-15. 1942.

Six examples of benign stromal endometriosis and one of clearly malignant nature are described. The concept that all instances of stromal endometriosis are benign is to be condemned as is also the interpretation of this form as low grade sarcoma. Adenomyosis uteri and stromal endometriosis are apparently variants of the same process, originating by extrusion of endometrium into myometrium.—H. G. W.

TUTA, J. A. [Grant Hosp., Chicago, Ill.] LYMPHANGIOENDOTHELIOMA OF THE UTERUS. Arch. Path., 33:78-83. 1942.

A rare case of lymphangioendothelioma of the uterus is reported.—H. G. W.

TUTA, J. A., and STUHR, W. A. [Grant Hosp., Chicago, Ill.] PRIMARY CARCINOMA OF THE FALLOPIAN TUBE. Am. J. Clin. Path., 11:864-870. 1941.

Two cases are reported, one operated without recurrence in 3 years.—H. G. W.

Ovary

BULFAMONTE, J. C. [Shamokin Hosp., Shamokin, Pa.] LARGE OVARIAN CYST IN A NEWBORN CHILD. Am. J. Surg., 55:175-176. 1942.

Report of a cystadenoma measuring 15 × 13 cm., removed by operation from a newborn child.—H. G. W.

FASQUELLE, E. [Hosp. Rosales, San Salvador, El Salvador] TUMOR DE KRUKENBERG. [KRUKENBERG TUMOR.] Arch. del Hospital Rosales, 69:81-88. 1941.

Illustrated report of a case.—M. D-R.

OTKEN, L. B. [Greenwood Leflore Hosp., Greenwood, Miss.] PRIMARY MELANOTIC SARCOMA OF THE OVARY. Am. J. Surg., 55:160-162. 1942.

A seventh case is added to the 6 reported as primary melanotic tumors of the ovary.—H. G. W.

Urinary System—Male and Female

PRIESTLY, J. T., and SCHULTE, T. L. [Mayo Clinic, Rochester, Minn.] THE TREATMENT OF WILMS' TUMOR. J. Urol., 47:7-10. 1942.

Of 39 patients traced since operation was performed in 1936 or before for Wilms' tumor, 6, or 15%, are living. In this series preliminary irradiation followed by nephrectomy and postoperative irradiation has given the best results.—H. G. W.

Oral Cavity and Upper Respiratory Tract

HOWES, W. E., and BERNSTEIN, L. [Brooklyn Cancer Inst., Brooklyn, N. Y.] ORAL CARCINOMA. Radiology, 37:468-475. 1941.

Carcinoma of the oral cavity is almost always of squamous cell origin and is divided by the authors into 4 histological types: intradermal, prickle cell, transitional cell, and anaplastic. Rare types are the basal cell and spindle, sarcoma-like varieties. Radiosensitivity may be estimated from the histological type, location, and size of a tumor as well as from the presence of ulceration, fungation, or infection.

One hundred and thirty-six cases seen at the Brooklyn Cancer Institute since 1936 are reviewed and tables are presented showing the age and sex incidence, location, and classification of the tumors. Ninety-three per cent of the patients were males and the highest incidence was found in the age group from 60 to 69 years. Among the possible etiological factors are heredity, changes due to syphilis, tuberculosis, arteriosclerosis, and vitamin deficiencies, chronic irritation from ill-fitting dentures or tooth fragments, and reactions to excessive use of tobacco and other irritants. Syphilis was present, by serological test, in over 25% of patients with carcinoma of the tongue, alveolar ridge, and buccal mucosa.

Treatment was almost entirely radiological, often with a combination of radium needles and roentgen therapy. The end results were poor in general, the highest 6-month survival rates being found in cancer of the tonsil (17% of 19 cases) and tongue (16% of 49 cases).—C. E. D.

Intrathoracic Tumors—Lungs—Pleura

GOLDMAN, A. [Univ. of California Hosp., San Francisco, Calif.] CARCINOMA OF THE LUNG OF LONG DURATION. J.A.M.A., 118:359-364. 1942.

A study of 11 cases of cancer of the lung of long duration, in this series averaging 90 months from the time of onset of symptoms until diagnosis. Ten of the 11 were epidermoid cancers, and the same proportion were peripheral instead of being in a main bronchus. The high incidence of cancer in men over 40 offers aid in differentiating the slowly growing tumors from adenoma, since nearly 2/3 of patients with adenoma have symptoms before 40 and the same proportion are female (12 of 19 patients). Exploratory thoracotomy to confirm the suspicion of pulmonary cancer is a logical procedure, and should be used as readily as an exploration for suspected abdominal cancer.—H. G. W.

HARRIS, W. H., and SCHATTENBERG, H. J. [Tulane Univ., Sch. of Med., New Orleans, La.] ANLAGEN AND "REST" TUMORS OF THE LUNG. New Orleans M. & S. J., 94:333-338. 1942.

The foregoing term is recommended by the authors for purpose of emphasis on the origin and histogenesis of certain lung tumors, in place of the term "mixed tumors" as applied by Womack and Graham.—H. G. W.

KORNBLUM, K., and BRADSHAW, H. H. [Jefferson Med. Coll. and Hosp., Philadelphia, Pa.] INTRATHORACIC NEUROGENIC TUMORS. Radiology, 37:391-406. 1941.

Case histories of 7 histologically verified intrathoracic neurogenic tumors are presented. Most of these tumors are found in the posterior part of the chest and may attain considerable size before giving rise to symptoms. Chest pain, cough, and weight loss are the most common complaints. Atelectasis, infection, pleural effusion, and phrenic nerve paralysis may occur as complications. Roentgenologically the tumors are generally dense, globular, and sharply circumscribed. They tend to displace rather than invade the thoracic organs and can usually be diagnosed roentgenographically.

Neurogenic tumors are highly radioresistant and surgical removal is the treatment of choice. No metastases were found in any of the authors' 7 cases and 2 patients were successfully treated surgically.—C. E. D.

ODESSKY, I. N. [Oustroumov Hosp. and 3rd Moscow State Med. Institute, Moscow, U. S. S. R.] THE ROENTGEN DIAGNOSIS OF NEURINOMA OF THE THORAX. Radiology, 37:454-457. 1941.

Three cases of neuroma of the chest wall are presented together with reproductions of roentgenographs.—C. E. D.

Heart

DEXTER, R., and WORK, J. L. [St. Alexis Hosp. and Sch. of Med. of Western Reserve Univ., Cleveland, Ohio] MYXOMA OF THE HEART. Arch. Path., 32:995-999. 1941.

A case of primary myxoma of the heart is described, which caused the signs and symptoms of mitral stenosis, and led to the death of a 53-year-old woman.—H. G. W.

Gastrointestinal Tract

EUSTERMAN, G. B. [Mayo Clinic, Rochester, Minn.] CARCINOMATOUS GASTRIC ULCER. J.A.M.A., 118:1-5. 1942.

Attention is called to the occasional healing changes produced by medical treatment of early carcinomatous ulcers, which leads to the error of considering them as benign.—H. G. W.

GOLDSTEIN, H. I. [Camden, N. J.] CANCER OF THE STOMACH IN CHILDREN AND YOUNG ADULTS. Rev. Gastroenterol., 8:450-458. 1941.

A report of a case seen in 1909, of a boy of 14, in which there were no gastric studies, no operation, and no autopsy, with a lengthy but uncritical review of the literature on gastric cancer in the young.—H. G. W.

HORSLEY, J. S. [St. Elizabeth's Hosp., Richmond, Va.] CARCINOMA OF THE JEJUNUM AND OF THE ILEUM. J. A. M. A., 117:2119-2123. 1941.

In a study of the literature from 1932 to January 1, 1941, 384 cases of carcinoma and carcinoid tumors of the jejunum and ileum have been found, of which 236 were adenocarcinoma of the jejunum or ileum, 7 were adenocarcinoma of Meckel's diverticulum, 138 were carcinoid of the jejunum or ileum, and 3 carcinoid of Meckel's diverticulum. Two cases were reported of what seemed to be primary chorioepithelioma of the small intestine. Two new cases of adenocarcinoma of the small intestine, with operation, are reported.—H. G. W.

LATIMER, E. O. [Wesley Memorial Hosp., Chicago, Ill.] MALIGNANT ARGENTAFFINE TUMORS OF THE APPENDIX. Am. J. Surg., 54:424-430. 1941.

While argentaffine tumors are most frequent in the appendix, being found in 0.2 to 0.5% of all appendices removed surgically, yet only 17 of the 76 malignant argentaffine tumors, including the 3 reported in this article, occurred in the appendix. Presumably this is because the appendiceal tumors produce symptoms earlier and are removed. All argentaffine tumors are potentially malignant.—H. G. W.

MARSHALL, S. F. [Lahey Clinic, Boston, Mass.] SUBTOTAL GASTRECTOMY IN A MAN EIGHTY-EIGHT YEARS OF AGE. Lahey Clinic Bull., 2:98-101. 1941.

A case of successful partial resection of the stomach for carcinoma in an 88-year-old man is described.—C. E. D.

VASS, A., and SIREA, D. M. [Springfield Hosp., Springfield, Ill.] LOCALIZED SUBACUTE PHLEGMONOUS GASTRITIS (SO-CALLED BOTRYOMYCOSIS) SIMULATING PYLORIC CARCINOMA. Am. J. Roentgenol., 46:59-68. 1941.

A case report with roentgenograms and photomicrographs.—C. E. D.

WALTERS, W., GRAY, H. K., and PRIESTLEY, J. T. [Mayo Clinic, Rochester, Minn.] MALIGNANT LESIONS OF THE STOMACH. J. A. M. A., 117:1675-1679. 1941.

A study of 11,000 patients with diagnosis of malignant disease of the stomach from 1907 to 1938 inclusive, in which 58% were subjected to surgical exploration, and in 26% the lesion was removed. The average mortality rate was 16%, whereas in 117 partial or total gastrectomies performed in 1940 the mortality rate was 8.8%. The 5-year survival rate after resection was 29%, and the 10-year survival rate was 20%. Of patients who did not have extension or metastases, 44.7% lived 5 years after leaving the hospital, and 17.3% of those having metastases lived 5 years. In 110, or 1.7% of the cases on which operation was performed, the lesion was sarcoma. Medical treatment of an ulcer type of dyspepsia should not be instituted without a roentgenological examination to determine the exact location and nature of the lesion.—H. G. W.

Liver

COATE, J. D. [Peralta Hosp., Oakland, Calif.] THE ROENTGENOLOGIC DEMONSTRATION OF TUMORS OF THE GALL BLADDER. Am. J. Roentgenol., 46:329-335. 1941.

Papillomas of the gall bladder are often multiple. They may occur in any region but are not usually found in the fundic pole. Adenomas are usually solitary and almost invariably are found in the extreme tip of the fundus, often associated with calculi. Roentgenologic diagnosis may be made by the finding of filling defects of constant location best seen as the gall bladder contracts following the fat meal. These tumors must be distinguished from radiolucent stones.

Eight case histories are presented, together with 12 roentgenograms, 4 photographs of surgical specimens, and 2 photomicrographs. Clinically these tumors are silent and symptoms arise from associated cholecystic disease. Adenomas of the gall bladder may be considered potentially malignant.—C. E. D.

THORSNESS, E. T. [Denver General Hosp., Denver, Colo.] THE RELATIONSHIP OF THE "TRUE LUSCHKA DUCTS," ADENOMAS, AND ABERRANT LIVER TISSUE IN THE WALL OF THE HUMAN GALL BLADDER. Am. J. Clin. Path., 11:878-881. 1941.

Case report.—H. G. W.

Liver

BERK, J. E., and LIEBER, M. M. [Jefferson Med. Coll., Philadelphia, Pa.] PRIMARY CARCINOMA OF THE LIVER IN HEMOCHROMATOSIS. Am. J. M. Sc., 202:708-714. 1941.

Primary carcinoma of the liver occurs with surprising frequency in hemochromatosis, 7.3%, as compared to an incidence of 4.5% in cirrhosis without hemochromatosis, and is apparently due to other factors found in hemochromatosis and not present in simple cirrhosis.—H. G. W.

COLVIN, S. H. [New Orleans, La.] PRIMARY CARCINOMA OF THE LIVER. New Orleans M. J., 94:242-243. 1941.

Case report.—H. G. W.

KIRSCHBAUM, J. D., and KOZOLL, D. D. [Cook County Hosp., Chicago, Ill.] CARCINOMA OF THE GALL BLADDER AND EXTRAHEPATIC BILE DUCTS. Surg., Gynec. & Obst., 73:740-754. 1941.

A clinical and pathological study of 117 cases occurring among 1,808 cases of malignant disease in 13,330 necropsies. Corrected figures show that 76.4% were in the female, and 66% in the white race. The youngest case was 42 years old. There were 55 cases of carcinoma of the gall bladder and 62 of the extrahepatic bile ducts. Of the 55 cases of gall bladder carcinoma, 36.3% gave evidences of cholecystitis and in 72.6% there was cholelithiasis. In 3% of the calculous gall bladders malignancy was present and in 30.6% of the 62 cases of bile duct cancer gall stones were found. An infiltrating type of adenocarcinoma is most frequent, with papillary adenocarcinoma next. Biliary cirrhosis is the most common complication. Pain was present in 60% and jaundice in a similar proportion of the gall bladder cancer cases, as compared with pain in 42% and jaundice in 92% of the bile duct cancers. Courvoisier's law seems to be verified more often by post-mortem examination than by clinical findings. A distended gall bladder was found in 29% of cases of gall bladder cancer and in 62.9% of duct cancer.—H. G. W.

Adrenal

KEPLER, E. J., and KEATING, F. R. [Mayo Clinic, Rochester, Minn.] DISEASES OF THE ADRENAL GLANDS. TUMORS OF THE ADRENAL CORTEX, DISEASES OF THE ADRENAL MEDULLA AND ALLIED DISTURBANCES. Arch. Int. Med., 68:1010-1036. 1941.

Part of a review on adrenal diseases, difficult to abstract, but of much value.—H. G. W.

Pancreas

VAYO, P. G., and BODON, G. T. [St. Mary's Hosp., Rochester, N. Y.] ADENOMA OF LANGERHANS' ISLET OF THE PANCREAS. Am. J. Surg., 54:744-746. 1941.

A case report of an adenoma of the islets with hypoglucemia, relieved by operative removal.—H. G. W.

Pituitary

RAKOFF, A. E., CANTAROW, A., and PASCHKIS, K. E. [Jefferson Med. Coll., Philadelphia, Pa.] CUSHING'S SYNDROME. J. Clin. Endocrinol., 1:912-915. 1941.

Two cases are studied with regard to estrogens, androgens, gonadotropins, pregnandiol, and adrenotropins. Benefit from treatment with stilbestrol was claimed in one case, and temporary amelioration in the other patient.—J. B. H.

Thymus

HELLWIG, C. A. [St. Francis Hosp. Wichita, Kans.] MALIGNANT THYMOMA. Surg., Gynec. & Obst., 73:851-863. 1941.

A report of 8 cases, of which 4 were lymphosarcoma, 1 lymphoid leucosarcomatosis, 2 Hodgkin's disease, and 1 thymic carcinoma. There is no clinical or roentgenological sign to differentiate between the different types, and no cure has been obtained by radiation.—H. G. W.

Thyroid

FRIEDELL, M. T. [Mayo Clinic, Rochester, Minn.] HYPERTHYROIDISM AND ADENOCARCINOMA OF THE THYROID GLAND. Arch. Surg., 43:386-396. 1941.

Out of 412 histologically proved and graded adenocarcinomas of the thyroid gland, a diagnosis of hyperthyroidism was made clinically in 57, or 13.8%. The incidence was highest in the fifth decade of life and in women. The authors found that hyperthyroidism was associated more frequently with adenocarcinomas of low malignancy, and they believe that at times these tumors may perform the function of benign thyroid tissue. They also found that as the grade of malignancy increases, the frequency of multiple adenomas decreases, but about one-half of the adenocarcinomas associated with hyperthyroidism occurred in glands containing multiple adenomas. In 24 cases, however, hyperthyroidism was present without the presence of multiple adenomas. The author concludes that this study supports the hypothesis of Ewing who states that an anaplastic tumor should retain less of the normal function of its constituent cells as the degree of anaplasia advances.—G. De B.